Miltenyi Biotec

MACSelect™ Transfected Cell Selection

User manual

MACSelect 4 MicroBeads	130-070-101
MACSelect K^k MicroBeads	130-070-201
MACSelect LNGFR MicroBeads	130-091-330
MACSelect LNGFR – Transfected Cell Selection Kit	130-091-879
pMACS 4.1	130-091-886
pMACS LNGFR-IRES	130-091-887
pMACS 4-IRES.II	130-091-888
pMACS K^k.II	130-091-889
pMACS LNGFR	130-091-890
MACSelect K^k – Transfected Cell Selection Kit	130-091-986
MACSelect 4 – Transfected Cell Selection Kit	130-091-988

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091-001-381-10

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1. Description

1.1 MACSelect™ Reagents

For a list of components please see enclosed attachment.

Size
For 25 separations

Storage
Store MicroBeads and FITC-conjugated antibodies protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label. Dissolve pMACS plasmid DNA in sterile, distilled water (use 25 µL for a concentration of 1 µg/µL or any other amount depending on the transfection method). Store dissolved DNA between –20 °C and –80 °C.

1.1.1 MACSelect MicroBeads

MACSelect MicroBeads are used for enrichment of transfected cells:
MACSelect K^k, 2 mL
MACSelect 4, 2 mL
MACSelect LNGFR, 2 mL

1.1.2 MACSelect Vectors

MACSelect Vectors are used to express a surface marker. To obtain a sufficient quantity of DNA for transfection, pMACS vectors can be amplified in *E.coli* for plasmid DNA preparation.

MACSelect Vectors for cloning of gene-of-interest (tube with green lid):
pMACS 4-IRES.II, 25 µg, lyophilized, for cloning and bicistronic expression of gene-of-interest together with truncated CD4 surface marker (not to be used for cotransfection).
pMACS LNGFR-IRES, 25 µg, lyophilized, for cloning and bicistronic expression of gene-of-interest together with truncated LNGFR surface marker (not to be used for cotransfection).

MACSelect Vector for cloning of gene-of-interest or cotransfection (tube with yellow lid):
pMACS K^k.II, 25 µg, lyophilized, for cotransfection or for cloning and expression of gene-of-interest and truncated H-2K^k surface marker from single vector.

MACSelect Vectors for cotransfection (tube with purple lid):
pMACS 4.1, 25 µg, lyophilized, for cotransfection.
pMACS LNGFR, 25 µg, lyophilized, for co-transfection.

MACSelect Control Vector for cotransfection (tube with red lid):
pMACS 14.1, 25 µg, lyophilized, control plasmid for set-up of cotransfection.

1.1.3 Fluorochrome-conjugated antibodies

Fluorochrome-conjugated antibodies are used to control the efficiency of transfection and enrichment.

Anti-H-2K^k-FITC, 0.25 mL or 1 mL, for analysis of transfection efficiency by labeling cells, that are not labeled with MACSelect H-2K^k MicroBeads

CD4-FITC, 0.25 mL or 1 mL, for analysis of transfection efficiency by labeling cells, that are not labeled with MACSelect 4 MicroBeads

CD271 (LNGFR)-FITC, 0.25 mL or 1 mL, for analysis of transfection efficiency by labeling cells, that are not labeled with MACSelect LNGFR MicroBeads

MACSelect Control-FITC, 0.25 mL or 1 mL, for analysis of enrichment by labeling transfected, MicroBead labeled cells

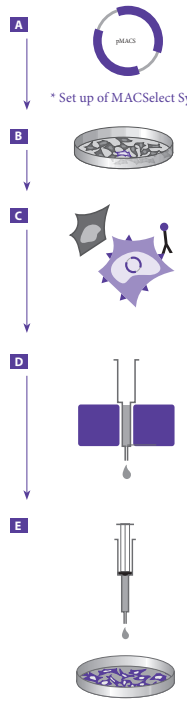
CD14-FITC, 0.25 mL or 1 mL, for analysis of control experiment (cotransfection)

1.2 Principle of MACSelect Transfected Cell Selection

The MACSelect System enables the enrichment of transfected cells. For MACSelect Enrichment, a surface marker is co-expressed with the gene-of-interest. Following transfection, only transfected cells express the marker. In the next step, these transfected cells are labeled with paramagnetic MicroBeads, that are coupled to monoclonal antibodies binding to the respective marker. MACS® Column Technology then enables the magnetic enrichment of the transfected, labeled cells.

For MACSelect Enrichment, the surface markers CD4, H-2K^k, or LNGFR are used. All pMACS vectors encode cytoplasmatically truncated surface markers to prevent signal transduction. They can be used for cotransfection in combination with an expression vector containing the gene-of-interest, or for cloning the gene-of-interest. Following cell transfection with any common method, transfected cells expressing the marker are magnetically labeled with MACSelect MicroBeads and separated using a MACS Separator and Separation Columns. Isolated cells can directly be taken into culture as the small MicroBeads are biodegradable and non-toxic. Alternatively, selected cells can be analyzed by flow cytometry, used for Western blot analysis, immunoprecipitation, reporter gene assays, RNA and DNA analysis, and many other applications.

1.2.1 MACSelect Single-Vector Transfection procedure



A *Clone gene-of-interest into pMACS vector.*

B *Transfect cells using any transfection method.*
Transfection control: Stain aliquot of cells with Anti-surface-marker-FITC.

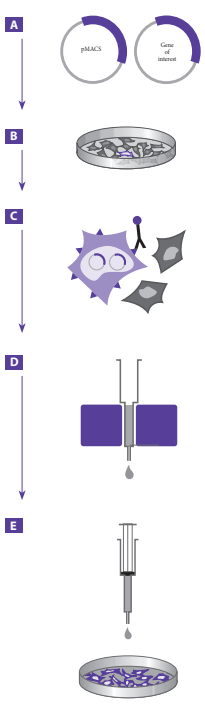
C *Label transfected cells with MACSelect MicroBeads.*
Enrichment control: Stain aliquot of cells (original fraction) with MACSelect Control FITC.

D *Load cell suspension on MACS® Column in MACS Separator and wash away unlabeled cells.*
Enrichment control: Stain aliquot of cells from collected flow-through and wash (negative) with MACSelect Control FITC.

E *Elute cells isolated with MACS Technology and culture cells or use cells for any downstream experiment.*
Enrichment control: Stain aliquot of eluted cells with MACSelect Control FITC.

*** Set up of MACSelect System**
• Determine best selection time (2.1)
1. Transfect cells with pMACS containing gene-of-interest.
2. Take aliquots of transfected, cultured cells at various time points.
3. Stain cell aliquots with Anti-surface-marker-FITC and analyze using microscopy or flow cytometry.

1.2.2 MACSelect Cotransfection procedure



A *Use pMACS and gene-of-interest expression vectors.*

B *Co-transfect cells using any transfection method.*
Transfection control: Stain aliquot of cells with Anti-surface-marker-FITC.

C *Label transfected cells with MACSelect MicroBeads.*
Enrichment control: Stain aliquot of cells (original fraction) with MACSelect Control FITC.

D *Load cell suspension on MACS Column in MACS Separator and wash away unlabeled cells.*
Enrichment control: Stain aliquot of cells from collected flow-through and wash (negative fraction) with MACSelect Control FITC.

E *Elute cells isolated with MACS Technology and culture cells or use cells for any downstream experiment.*
Enrichment control: Stain aliquot of eluted cells with MACSelect Control FITC.

*** Set up of MACSelect System**
• Establish best cotransfection ratio (see 2.2)
1. Cotransfect cells with pMACS and gene-of-interest expression vectors in different molar ratios (refer to 2.2 and 3.1).
2. Analyze gene-of-interest and surface marker expression.
• Determine best selection time (refer to 2.1)
1. Cotransfect cells with pMACS and gene-of-interest expression vectors.
2. Take aliquots of transfected, cultured cells at various time points.
3. Stain cell aliquots with Anti-surface-marker-FITC and analyze using microscopy or flow cytometry.
• Optional: Control experiment for cotransfection and enrichment (see 2.3)

1.3 Background and product applications

MACSelect - Transfected Cell Selection Systems enable the fast enrichment of transfected cells without antibiotic treatment. They can be used for any cultured or primary cell type, whether adherent or in suspension, using any commonly used transfection method.
The enrichment rate of transfected cells is influenced by the experimental design, and may vary depending on cell type and transfection conditions used.
MACSelect System enables a stable cell line to be created by repeated magnetic enrichment. MACS Technology allows selected cells to be analyzed directly using flow cytometry, or studied using Western blot analysis, immunoprecipitation, reporter gene assays, RNA and DNA analysis and many other applications. Alternatively, isolated cells can directly be taken into culture as the small MicroBeads are biodegradable and non-toxic.

Examples of applications

- Enrichment of a homogenous cell culture population of transiently transfected cells, thus eliminating the need for setting-up stable cell lines.[1, 2, 3]
- Enrichment of a homogenous, transiently transfected primary cell culture to a percentage which enables most downstream experiments.[4, 5]
- Fast, efficient method to establish stably transfected cell lines.[6]
- Selection of retrovirally transduced primary cells.[7]
- Selection of transfected cells when a rapid, nontoxic, and nonimmunogenic method is necessary.[8]
- Selection of transduced T cells, other hematopoietic cells or stem cells, for example for research on allogeneic hematopoietic stem cell transplantations.[8]

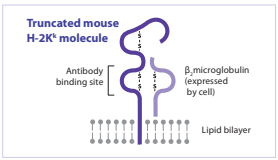
1.4 MACSelect System choice

1.4.1 MACSelect Surface Markers

MACSelect - Transfected Cell Selection Systems use pMACS vectors that are designed to express a particular cell surface marker, such as CD4, H-2K^k, or LNGFR. All MACSelect surface marker genes encoded by pMACS vectors are truncated in their cytoplasmatic domain in order to prevent any signal transduction through the respective receptor.

MACSelect K^k selection marker
The MACSelect K^k selection marker is a mouse MHC class I H-2K^k protein with a truncated cytoplasmatic domain. H-2K^k requires the co-expression of β2-microglobulin on the cell surface.

! Note: H-2K^k expression is restricted to some rarely used mouse strains like AKR/J and CBA/Ca. The MACSelect K^k system is not suitable for such cell types.

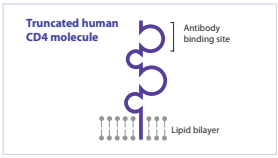


MACSelect K^k Tag Vector Sets
are available for the enrichment of transfected cells expressing a c-myc-, HA-, or His-tagged protein of interest.

MACSelect 4 selection marker
The MACSelect 4 selection marker is a human CD4 antigen with a truncated cytoplasmatic domain.

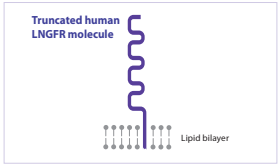
! Note: CD4 is naturally expressed on human T helper cells, monocytes and dendritic cells. The MACSelect 4 system is not suitable for such cell types of human origin.

! Note: The CD4 surface protein is sensitive to trypsin, therefore the MACSelect 4 system cannot be used with cells that need to be detached with trypsin (usually EDTA is sufficient).



MACSelect LNGFR selection marker
The MACSelect LNGFR selection marker is a human, low-affinity nerve growth factor receptor. LNGFR is also known as CD271, NGF receptor, p75 ICD, low affinity neurotrophin receptor or tumor necrosis factor receptor superfamily member 16 precursor.

! Note: LNGFR is expressed in the central and peripheral nervous system on autonomic and sensory neurons, but also on glial cells including oligodendrocytes, astrocytes, and Schwann cells. Additionally, LNGFR can be found on bone marrow fibroblasts, follicular dendritic cells, and mesenchymal cells involved with mesenchymal-epithelial interactions. The MACSelect LNGFR system is not suitable for such cell types of human origin.



1.4.2 Cotransfection versus single-vector transfection

The MACSelect System offers the choice between cotransfection or single-vector transfection.
Cotransfection: The optimal cotransfection ratio of pMACS and the individual gene-of-interest expression vector has to be determined in a set up experiment, (see 2. "Set up of the MACSelect System"). pMACS 4.1, pMACS K^k.II, and pMACS LNGFR are used for cotransfection with an expression vector containing the gene-of-interest. The co-transfection ratio is usually higher than 90%.[9]

Single-vector transfection: The gene-of-interest has to be cloned into the multiple cloning site of the respective pMACS vector. pMACS 4-IRES.II, pMACS K^k.II, pMACS LNGFR-IRES, and pMACS K^k.Tag (c-myc/HA/His; N/C) are available for this purpose. Vectors with an IRES (Internal Ribosome Entry Site) sequence produce a bicistronic transcript, that allows the expression of two different proteins from a single mRNA. These IRES containing vectors should only be used when a gene-of-interest is cloned into the multiple cloning site.
Vectors with a tag sequence enable the expression of a N- or C-terminal-tagged protein of interest for downstream protein isolation or analysis.
MACSelect – Transfected Cell Selection Systems can be used with all commonly used transfection methods.

co-transfection	single-vector transfection
pMACS 4.1 pMACS K^k.II pMACS LNGFR in combination with an individual gene-of-interest expression vector	pMACS 4-IRES.II pMACS K^k.II pMACS LNGFR-IRES pMACS K^k.His (N/C) pMACS K^k.HA (N/C) pMACS K^k.c-myc (N/C)
control vector pMACS 14.1 for set up (see 2. Set up of the MACSelect™ System)	
no cloning, direct start with plasmid preparation and transfection	clone gene-of-interest into multiple cloning site of pMACS vector
expression of gene-of-interest and surface marker from two different plasmids	expression of two proteins from one vector (bicistronic expression with IRES vector or expression from two different promoters)
most flexibility regarding expression time by establishing best fitting co-transfection ratios	no need to establish optimal transfection ratio of co-transfection

1.5 Related products

MACSelect His Vector Set	130-092-083
MACSelect HA Vector Set	130-092-084
MACSelect c-myc Vector Set	130-092-085

1.6 Reagent and instrument requirements

- PBS: Phosphate buffered saline, pH 7.2, without EDTA (3.2.1. "Procedure A")
- PE: PBS with 5 mM EDTA (3.2.2 "Procedure B")
- PBE: PBS supplemented with 0.5% bovine serum albumin (BSA) and 5 mM EDTA.
  ! Note: Use degassed PBE only! Excess of gas in PBE will form bubbles in the matrix of the column during separation. This may lead to clogging of the column and may decrease the quality of separation.
- Reagents for cloning of gene-of-interest (single-vector transfection only)
- Reagents for plasmid amplification
- Reagents for transfection of cells and appropriate cell culture medium.
- For 3.2.3 "Procedure C" only (not for MACSelect 4):
  Trypsin solution: 0.05% trypsin dissolved in PBS with 2 mM EDTA,
  100% FCS.
- Cell counting device, e. g. hemacytometer.
- MACS® Columns and MACS Separators:

Column	Max. number of labeled cells	Max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS VarioMACS, SuperMACS
autoMACS	2×10^8	4×10^9	autoMACS

! Note: Column adapters are required to insert columns into VarioMACS™ or SuperMACS™ Separator. For details, see MACS Separator data sheets.

1.7 General tips & hints

- **Centrifuge cells** at 50–200×g depending on cell type: the larger the cells are, the lower the centrifugation speed should be. Many adherent cells are rather large and should be centrifuged at a maximum speed of 50–100×g. Higher speeds may cause them to burst. The centrifugation speed of suspension cells should not exceed 200×g.
- Many adherent cells can be detached with **EDTA only**.
- A typical transfection can result in up to 50% **dead cells**. Dead cells may bind non-specifically to MACSelect MicroBeads or cause separation columns to clog. If transfected adherent cells are to be selected, the non-adherent dead cells should be removed by washing before harvesting the cells. For efficient enrichment of suspension cells, we recommend depleting dead cells prior to magnetic labeling. Very gentle and fast removal of dead cells can be achieved using MACS Dead Cell Removal Kit (# 130-090-101, see Appendix 6.1).
- For proper performance of the MACSelect-Transfected Cell Selection system it is very important to obtain a **single-cell suspension** before magnetic separation. Cell doublets or aggregates must be separated by carefully pipetting the cells up and down using a 1 mL pipette tip. Generation of foam should be avoided. If transfected adherent cells are to be selected, we recommend harvesting non-confluent cell cultures to achieve single-cell suspensions. The single-cell suspension should be checked using a microscope.

2. Set-up of the MACSelect System

The efficiency of transfected cell selection is influenced by various experimental parameters, such as the cell type and culture conditions, the transfection method and, in the case of cotransfection, the vector ratios. Therefore, we recommend initially determining the time of highest selection marker expression and - in case of cotransfection - optimizing the plasmid vector ratios for the transfection before using the MACSelect System.

! Note: The use of plasmids encoding green fluorescent protein (GFP) for establishing the MACSelect System is not recommended. Most commercially available plasmids express GFP from the extremely strong CMV promoter with an open reading frame mutated for optimized codon usage. Therefore, GFP expression often does not correlate with the expression level of other proteins. Additionally, GFP accumulates in the cytoplasm so that even minor quantities of GFP will show a green fluorescent signal.

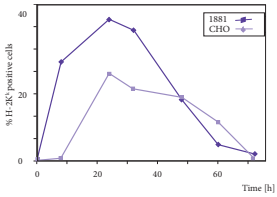
2.1 Determination of the best selection time

Following transfection, cells should be cultured for a short time to express the MACSelect surface marker before being magnetically enriched. With different cell types, transfection methods, and culture conditions, the expression kinetics of the CD4, H-2K^k, or LNGFR selection marker differ. Therefore, the time of highest marker expression, which is the best selection time, needs to be determined for the individual experiments. Aliquots of cells can be stained with fluorochrome-conjugated antibodies recognizing the MACSelect surface marker following cell transfection (the respective FITC-labeled antibodies are included in MACSelect Kits and are available separately). The expression kinetics of the FITC-labeled selection marker can be analyzed using microscopy or flow cytometry analysis.
Depending on the transfection method, the following culture periods after transfection and before staining are recommended:

- electroporation: 6–48 h cell culture
- lipofection: 24–60 h cell culture
- calcium phosphate precipitation: 48–84 h cell culture

Generally, by analyzing 3 time points after transfection the optimal selection time can be defermined. Time points should be chosen according to the cell division rate and transfection method used. For electroporated cells with a standard division rate, time points of approximately 16h (±1h), 20h (±1h), and 24h (±1h) can be chosen. For quickly dividing cells (e.g. 1881, Jurkat) these should be 6h (±1h), 14h (±1h), and 18h (±1h) after electroporation.

The figure below shows an example of pMACS K^k.II expression kinetics in different cell lines (for further examples please refer to Appendix 6.2).



Optional: If the expression maximum of the gene-of-interest can additionally be determined (via fluorochrome detection, enzymatic assay, etc.), the optimal time point for selection can be adjusted accordingly: if the selection marker is expressed earlier than the gene-of-interest, the cells should be enriched at the time of highest selection marker expression. After enrichment, they should be taken back into culture until the expression of the gene-of-interest reaches its maximum. If the expression of the gene-of-interest peaks before the expression of the selection marker, the cells should be enriched when both genes are equally well expressed.

1. Transfect cells (according to 3.1).
   ! Note: To determine the best time of selection, we recommend transfecting the cells with the amount of DNA that will also be used in further experiments.
2. After transfection, culture cells for the appropriate time period (see information above).
3. Continue with 4.2 "Staining of cells with fluorochrome-conjugated antibodies" to visualize surface marker expression.

2.2 For cotransfection only: establishing the optimal cotransfection ratio

To establish the optimal cotransfection ratio of pMACS vector and gene-of-interest expressing vector, cells should be co-transfected with a minimum of three different vector ratios. Excess of gene-of-interest vector ensures that cells enriched with MACSelect System contain the gene-of-interest. pMACS:gene-of-interest molar vector ratios of 1:1.5, 1:2.5, and 1:5 are recommended. To analyze gene expression, we recommend fluorescently double staining of aliquots of the cells following transfection with the respective fluorochrome-labeled anti-surface marker and anti-gene-of-interest antibody and subsequent microscopic or flow cytometric analysis.

Alternatively, gene-of-interest expression can be monitored with an enzymatic assay or other methods. The optimal cotransfection ratio shows a high surface marker expression while ensuring that every cell expressing surface marker also expresses the gene-of-interest.

2.3 Control experiment for cotransfection

For a convenient analysis of cotransfection, the control vector pMACS 14.1 is included in MACSelect kits. pMACS 14.1 encodes human CD14 as the gene-of-interest. This CD14 expression allows fluorescent monitoring of control gene expression by using CD14-FITC (included in MACSelect Kits).

! Note: Human monocytic cells or cell lines expressing CD14 are not suitable for transfection with pMACS 14.1 (e.g. U937 cells). Some cell lines do not express a glycophosphoinositol linker so that CD14 expression in these cells is not detectable (e.g., mouse L-cells or some Raji cells).

1. Co-transfect cells with pMACS 14.1 and the pMACS cotransfection vector (e.g. pMACS K^k.II, pMACS 4.1, pMACS LNGFR) using a standard transfection protocol. Use 1–10 µg of the pMACS plasmid depending on the transfection method and the type of cells used.
   ! Note: The SV40 promoter may be downregulated in some lymphocytic cell lines. To compensate for the lower promoter activity, the ratio of pMACS 14.1 should be increased.
2. Enrich the transfected cells using the protocols in section 3.2. and 3.3.
3. Analyze transfection and enrichment efficiency with 4.2 "Staining of cells with fluorochrome-conjugated antibodies" by using Anti-H-2K^k-FITC, CD4-FITC, or CD271 (LNGFR)-FITC, and CD14-FITC in parallel.

3. Enrichment of transfected cells using the MACSelect™ System

3.1 Transfection of cells

Before starting: Prepare plasmid DNA by amplification in *E.coli* and purification.

1. Transfect the cells according to a standard transfection protocol
   ! Note: Use the transfection protocol for the individual transfection reagent or a protocol established for the respective cells.
   ! Note: Detailed protocols for the transfection of adherent cells, e.g., CHO cells, using electroporation or lipofection are available. To receive further information, please contact your local Technical Service Team or visit **www.miltenyibiotec.com**.

For single-vector transfection:

Use 10–20 µg of the pMACS plasmid depending on the transfection method, the cell number, and the type of cells used.

For cotransfection:

Cotransfect cells with your plasmid construct and the pMACS plasmid using your standard transfection protocol. Use 1–10 µg of the pMACS plasmid depending on the transfection method and the type of cells used. Use your plasmid construct with the ratio determined in 2.2 "Establishing the optimal cotransfection ratio" or in an 1.5-fold molar excess.

2. After transfection, culture the cells for an appropriate period as determined in 2.1 "Determination of the best selection time".

3.2 Magnetic labeling

At the time point of optimal marker expression as determined in 2.1 "Determination of the best selection time", perform harvest and magnetic labeling of cells according to the procedures given below.

Adherent cells

- Gentle labeling and harvest of cells (no cell centrifugation)
  - 3.2.1 Procedure A: EDTA harvest of cells
  - 3.2.3 Procedure C: Trypsin harvest of cells
- Large cell culture vessels (> 250 mL flask)
  - 3.2.2 Procedure B: harvest of large numbers of cells

Non-adherent cells

- 3.2.2 Procedure B: harvest of non-adherent cells
- Special application
  - 3.2.4 Procedure D: harvest of transduced T cells

3.2.1 Procedure A: harvest and magnetic labeling of transfected adherent cells with EDTA

Before starting
Pre-cool PBS on ice.
Degas PBE and pre-cool on ice.

1. Remove medium from petri dish or flask.
2. Wash cells with PBS **without EDTA**.
   ! Note: This step removes dead cells which could interfere with separation performance.
3. Remove PBS completely.
4. Add **PBE** and **MACSelect MicroBeads** according to the table below.

Culture dish	ø 9 cm	ø 6 cm	250 mL flask (75 cm^2)
PBE	600 µL	300 µL	800 µL
MACSelect MicroBeads	80 µL	40 µL	110 µL

5. Rock gently to disperse and incubate for 15 minutes at 4–8 °C. Rock gently two more times during incubation.
6. Tap or shake dish or flask to detach cells completely.
7. Add PBE and adjust to a final volume of 2 mL and suspend the cells carefully to prepare a single cell preparation.
   ! Note: For the efficient enrichment of transfected cells, ensure that a very good **single-cell suspension** is prepared by carefully pipetting the cells up and down using a 1 mL pipette tip. **The single-cell suspension should be checked using a microscope.**
8. Proceed to 3.3 "Magnetic separation".

3.2.2 Procedure B: harvest and magnetic labeling of transfected non-adherent cells or large numbers of adherent cells

Before starting
Pre-cool PBS and PE (PBS supplemented with 5 mM EDTA) on ice.
Degas PBE and pre-cool on ice.

! Note: For efficient enrichment of suspension cells, we recommend depleting dead cells prior to magnetic labeling. Very gentle and fast removal of dead cells can be achieved using the MACS Dead Cell Removal Kit (# 130-090-101, see Appendix 6.1).

! Note: Volumes for magnetic labeling are indicated for 10^7 total cells. When working with fewer than 10^7 cells, use same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for $2x10^7$ total cells use twice the volume of all indicated reagent volumes and total volumes).

1. For adherent cells only: Wash transfected cells with PBS and harvest cells using PE.
2. Centrifuge at 50–200×g (depending on the cell type) for 10 minutes, carefully remove supernatant completely.
   ! Note: The larger the cells are, the lower the centrifugation speed should be.
3. Resuspend cell pellet in 320 µL of **PBE** per 10^7 total cells.
4. Add 80 µL of **MACSelect MicroBeads** per 10^7 total cells to magnetically label the transfected cells.
5. Mix well and incubate for 15 minutes on ice.
6. If the volume is below 2 mL: adjust to 2 mL by adding PBE.
7. Proceed to 3.3 "Magnetic separation".

3.2.3 Procedure C: Trypsin harvest and magnetic labeling of transfected adherent cells

Before starting
Degas PBE

1. Remove medium from petri dish or flask.
2. Wash cells with PBS **without EDTA**.
   ! Note: This step removes dead cells which could interfere with separation performance.
3. Remove PBS completely.
4. Add **trypsin solution** according to the table below.

Culture dish	ø 9 cm	ø 6 cm	250 mL flask (75 cm^2)
Trypsin solution	500 µL	250 µL	650 µL
FCS	100 µL	50 µL	150 µL
MACSelect MicroBeads	80 µL	40 µL	110 µL

5. Tap dish and incubate at room temperature until the cells have dissociated from the culture dish and from each other.
6. Stop trypsinization by adding 100 µL of **100% FCS** per 9 cm petri dish. For other cell culture dishes or flasks, use volumes of FCS according to the table above.
7. Add 80 µL of **MACSelect MicroBeads** per 9 cm petri dish. For other cell culture dishes or flasks, use volumes of MACSelect MicroBeads solution according to the table above.
8. Rock gently to disperse and incubate for 15 minutes at 4–8 °C. Rock gently two more times during incubation.
9. Add **PBE** and adjust to a final volume of 2 mL and resuspend the cells completely to prepare a single cell preparation.
   ! Note: For the efficient enrichment of transfected cells, ensure that a very good **single-cell suspension** is prepared by carefully pipetting the cells up and down using a 1 mL pipette tip. **The single-cell suspension should be checked using a microscope.**
10. Proceed to 3.3 "Magnetic separation".

3.2.4 Procedure D (special application): harvest and magnetic labeling of transduced T cells

Novel approaches in research on allogeneic hematopoietic stem cell transplantations utilize the strategy to modulate graft-versus-leukemia effects by introducing an inducible suicide gene and the intracytoplasmatic truncated cell surface marker LNGFR into allogeneic donor lymphocytes[8]. The protocol below has been exclusively developed for this special application.

Before starting
Pre-cool PBS on ice.
Degas PBE and pre-cool on ice.

! Note: LS Columns must be used.

! Note: Volumes for magnetic labeling are indicated for $4x10^7$ total cells. When working with fewer than $4x10^7$ cells, use same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly.

1. Centrifuge transduced cells at 100–200×g for 10 minutes, carefully remove supernatant completely.
   ! Note: The centrifugation speed of suspension cells should not exceed 200×g. Higher speeds may cause them to burst.
2. Resuspend cell pellet in 320 µL of **PBE** per $4x10^7$ total cells.
3. Add 80 µL of **MACSelect LNGFR MicroBeads** per $4x10^7$ total cells to magnetically label the transfected cells.
4. Mix well and incubate for 15 minutes on ice.
5. Add PBE and adjust to a final volume of 2 mL per $4x10^7$ total cells.
6. Proceed to 3.3 "Magnetic separation" on LS Column.

3.3 Magnetic separation

3.3.1 Magnetic separation with MS or LS Column

! Note: Choose an appropriate MACS® Column and MACS Separator according to the number of transfected cells (see table 1.5). When separating transduced T cells LS Columns must be used.

! Note: If the transfected cells are taken into culture after enrichment, we recommend using culture medium instead of PBE for the final elution step. All other magnetic separation steps must be performed using PBE in order to obtain highest enrichment results.

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of PBE:
   MS: 500 µL LS: 3 mL
3. Optional: For analysis of enrichment (see 4.1) remove an aliquot of the magnetically labeled, transfected cells for subsequent fluorescent staining **(original fraction)**.
4. Apply cell suspension in an appropriate amount of PBE onto the column:
   MS: 1–2 mL LS: 1–10 mL.
   ! Note: When working with adherent cells, apply the cells in aliquots of 500 µL onto the column. Each aliquot must be well resuspended before being applied to the column.
5. Collect unlabeled cells that pass through and wash with appropriate amount of PBE. Perform washing steps by adding buffer successively once the column reservoir is empty.
   MS: 4×500 µL LS: 4×3 mL.
   Collect total effluent. This is the unlabeled cell fraction **(negative fraction)**.
6. Remove column from the separator and place it on a suitable collection tube.
7. Pipette appropriate amount of PBE onto the column. Immediately flush out transfected cells by firmly applying the plunger, supplied with the column.
   MS: 1 mL LS: 5 mL.
   ! Note: To increase the purity of the magnetically labeled fraction pass the cells over a second, freshly prepared column.
8. Optional: For analysis of enrichment, remove an aliquot of the eluted cells for fluorescent staining **(positive fraction)**.

3.3.2 Magnetic separation with the autoMACS® Separator

! Note: Refer to the autoMACS® User Manual for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose the program "Posseld".
3. Collect the positive fraction from outlet port pos2.

4. Analysis of enrichment

4.1 Determination of MACSelect Enrichment rate

The MACSelect Enrichment rate of transfected cells can vary with the individual experimental design that is used (e.g., cell line and transfection method). Therefore it is advisable to determine the enrichment rate in initial experiments. To measure the enrichment, aliquots of magnetically labeled, transfected cells are fluorescently stained with MACSelect Control FITC Antibody. This fluorochrome-conjugated antibody specifically stains MACSelect MicroBead-labeled cells. By comparing the fluorescent staining of magnetically labeled cells before and after magnetic separation, the enrichment factor can be determined.

! **Note:** Do not use fluorochrome-conjugated antibodies recognizing a MACSelect Surface Markers (e.g., Anti-H-2K^k-FITC, CD4-FITC, CD271 (LNGFR)-FITC) as most surface receptor epitopes are blocked by MicroBeads.

! **Note:** The MACSelect Control FITC antibody can cross-react with B cells.

! **Note:** The use of plasmids encoding green fluorescent protein (GFP) for establishing MACSelect System is not recommended (see 2. "Set up of the MACSelect™ System").

1. Centrifuge the magnetically labeled, original, negative, and positive fractions for 5 minutes at 50–200×g (depending on the cell type) at room temperature.

! **Note:** The larger the cells are, the lower the centrifugation speed should be.

2. Remove the supernatant completely and resuspend the cells in 100 µL of PBE.

3. Add 10 µL of MACSelect Control FITC Antibody.

4. Mix well and incubate for 5–10 minutes in the dark at 4–8 °C.

5. Wash cells by adding 10–20× the staining volume of buffer and centrifuge at 50–200×g for 5 minutes at room temperature.

6. **Flow cytometric analysis:** Resuspend cells in an appropriate amount of buffer and proceed to analysis.
Alternatively, **fluorescence microscopic analysis:** Resuspend cell pellet without adding buffer and transfer resuspended cells to a glass slide. Cover the cells with a cover slip and proceed immediately to microscopic analysis.

! **Note:** Perform the microscopic evaluation directly after fluorescent staining. A delay of the analysis may result in a decrease in the fluorescent intensity.

The enrichment rate (f_E) represents the factor by which the ratio of surface marker positive cells versus negative cells is increased after the enrichment. Assuming that all positive cells are retained, the f_E represents the average number of negative cells that pass through the column per negative cells retained non-specifically. This value is low if cells are unspecifically labeled and retained.

$$f_E = \frac{\text{(\% neg. cells in orig. sample)}}{\text{(\% pos. cells in orig. sample)}} \times \frac{\text{(\% pos. cells in pos. fraction)}}{\text{(\% neg. cells in pos. fraction)}}$$

4.2 Staining of cells with surface marker specific fluorochrome-conjugated antibodies

! **Note:** Do not use fluorochrome-conjugated antibodies recognizing a MACSelect Surface Marker (e.g., Anti-H-2K^k-FITC, CD4-FITC, CD271 (LNGFR)-FITC) on magnetically labeled cells as most surface receptor epitopes are blocked by MicroBeads.

1. Centrifuge non magnetically labeled cells for 5 minutes at 50–200×g (depending on the cell type) at room temperature.

! **Note:** The larger the cells are, the lower the centrifugation speed should be.

2. Remove the supernatant completely and resuspend the cells in 100 µL of PBE.

3. Add 10 µL of FITC-conjugated antibody (e.g., Anti-H-2K^k-FITC, CD4-FITC, CD271 (LNGFR)-FITC, or CD14-FITC).

4. Mix well and incubate for 5–10 minutes in the dark at 4–8 °C.

5. Wash cells by adding 10–20× the staining volume of buffer and centrifuge at 50–200×g for 5 minutes at room temperature.

6. **Flow cytometric analysis:** Resuspend cells in an appropriate amount of buffer and proceed to analysis.
Alternatively, **fluorescence microscopic analysis:** Resuspend cell pellet without adding buffer and transfer resuspended cells to a glass slide. Cover the cells with a cover slip and proceed immediately to microscopic analysis.

! **Note:** Perform the microscopic evaluation directly after fluorescent staining. A delay of the analysis may result in a decrease in the fluorescent intensity.

5. Troubleshooting

Low enrichment

- For magnetic separation, make sure that a **single-cell suspension** is loaded in **aliquots** onto the MACS Column. Presence of cell doublets or aggregates can be checked by microscopy. Cell aggregates or doublets lead to the co-enrichment of negative cells.
- Use the optimal time point for enrichment (refer to 2. "Set up of the MACSelect™ System").
- In case a very low number of positive cells should be enriched, separating the eluted, positive fraction on a second column may increase purities. Therefore repeat 3.3.1 "Magnetic separation with MS or LS Column".
- For pMACS vectors with gene-of-interest: ensure correct cloning of vector. For pMACS 4-IRES.II and pMACS LNGFR-IRES: make sure that the gene-of-interest is cloned without a polyA signal sequence into MCS.
- For co-transfection only: By 2.2 "Establishing the optimal co-transfection rate" pMACS vector amount should be chosen such that surface marker expression is sufficient for MACSelect Enrichment.
- To control the MACSelect Procedure, perform co-transfection control experiment with pMACS 14.1.

Gene-of-interest expressing cells in negative fraction

- When using a bicistronic vector (such as pMACS 4-IRES.II or pMACS LNGFR-IRES), the expression of the gene-of-interest is much stronger than the expression of the surface marker. Accordingly, some cells showing a weak gene-of-interest expression but no surface marker expression can be detected in the flow-through/wash fraction.
- When performing a co-transfection, the gene-of-interest vector should be used in a molar excess compared to the pMACS vector so that any surface marker expressing cell also expresses the gene-of-interest. Accordingly, some cells might show a gene-of-interest expression but only weak surface marker expression.

Negative cells in positive fraction

- For magnetic separation, make sure that a **single-cell suspension** is loaded in **aliquots** onto the MACS Column. Presence of cell doublets or aggregates can be checked by microscopy. Cell aggregates or doublets lead to the co-enrichment of negative cells.

Column clogging

- A high number of dead cells, or genomic DNA in the cell suspension, can lead to column clogging. When enriching suspension cells, the MACS Dead Cell Removal Kit should be used. For adherent cells, perform washing steps as denoted in the protocol. Additionally, perform slower cell centrifugation steps.
- Use degassed buffer only! Excessive gas in the running buffer will form bubbles in the matrix during isolation. Air bubble formation in the MACS Column may lead to clogging of the column and decrease the quality of the isolation. This is particularly important when the applied buffer has a different temperature to that of the column, e.g. when using cold buffer on a column at room temperature. Degas buffer by applying vacuum or sonification for ten minutes, preferentially with the buffer at room temperature.

Weak staining with fluorochrome-conjugated antibodies

- Do not use antibodies recognizing MACSelect Surface Markers on MicroBead-labeled cells: many epitopes recognized by the fluorochrome-conjugated antibody will already be covered by the respective antibodies on the MicroBeads. Use MACSelect Control FITC Antibody to stain MicroBead-labeled cells.

6. Appendix

6.1 Removal of dead cells using MACS Dead Cell Removal Kit

! **Note:** Choose an appropriate MACS Column and MACS Separator according to the number of transfected cells (see 1.5 "Reagent and instrument requirements").

1. Prepare 1× Binding Buffer: per 10^7 **total cells**, dilute 0.25 mL of 20× Binding Buffer stock solution with 4.75 mL of sterile, distilled water.

2. Harvest transfected cells after an appropriate incubation period.

3. Centrifuge cells at 200×g. Remove supernatant completely and resuspend cell pellet in 100 µL of Dead Cell Removal MicroBeads per 10^7 total cells. For fewer cells, use same volume. Mix well and incubate for 15 minutes at **room temperature** (20–25 °C).

4. Prepare column by washing with 1× Binding Buffer: MS: 500 µL LS: 3 mL.

! **Note:** Alternatively: use autoMACS® Separator and proceed to 8–9.

5. Apply cell suspension in a suitable amount of 1× Binding Buffer onto the column:
MS: 500–1000 µL LS: 1–10 mL.

6. Allow the living cells to pass through. Rinse with an appropriate amount of 1×Binding Buffer: MS: 4×500 µL LS: 4×3mL.

7. Collect effluent as live cell fraction and proceed to 3.2 "Magnetic labeling" of the transfected cells.

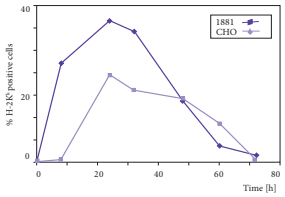
8. Prepare and prime autoMACS Separator.

! **Note:** Refer to the autoMACS User Manual.

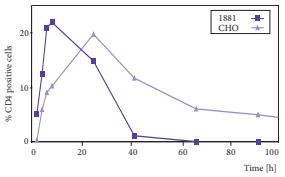
9. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose the program "Possel". Collect the positive fraction from outlet port pos1 and proceed to 3.2 "Magnetic labeling".

6.2 Examples of pMACS expression kinetics in different cell lines

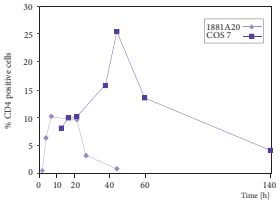
Example of pMACS K^k.II expression kinetics in different cell lines
Chinese hamster ovarian cells (CHO) or the mouse pre-B cell line 1881 were transfected with pMACS K^k.II by electroporation. Transfected cells were fluorescently stained at various times using H-2K^k-FITC and analyzed by flow cytometry.



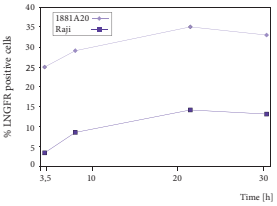
Example of pMACS 4.1 expression kinetics in different cell lines
The mouse pre-B cell line 1881 and Chinese hamster ovarian cells (CHO) were transfected with pMACS 4.1 by electroporation. Transfected cells were fluorescently stained at various times using CD4-FITC and the percentage of fluorescent cells was determined by flow cytometry.



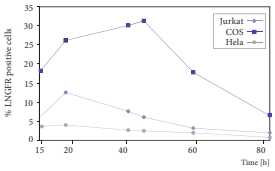
Example of pMACS 4-IRES.II expression kinetics in different cell lines
African green monkey kidney cells (COS 7) or the mouse pre-B cell line 1881 were transfected with pMACS 4-IRES by electroporation. Transfected cells were fluorescently stained at various times using CD4-FITC and analyzed by flow cytometry.



Example of pMACS LNGFR expression kinetics in different cell lines
The mouse pre-B cell line 1881 and human B cells (Raji) were transfected with pMACS LNGFR by electroporation. Transfected cells were fluorescently stained at various times using CD271 (LNGFR)-FITC and the percentage of fluorescent cells was determined by flow cytometry.



Example of pMACS LNGFR-IRES expression kinetics in different cell lines
The human T lymphocyte cell line Jurkat (Jurkat), the African green monkey kidney cell line COS-7 (COS) and the human, epithelial, adenocarcinoma cell line HeLa (HeLa) were transfected with pMACS LNGFR by electroporation. Transfected cells were fluorescently stained at various times using CD271 (LNGFR)-FITC and the percentage of fluorescent cells was determined by flow cytometry.

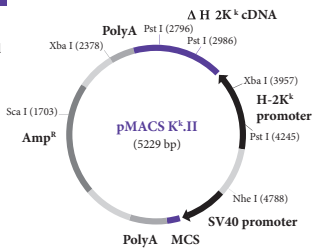


6.3 pMACS vector information and maps

The following pMACS vectors encode an ampicillin resistance gene and can be amplified by transformation in commonly used *E. coli* strains such as Top10. Standard plasmid DNA purification methods that yield transfection-quality DNA can be used, e.g., by DEAE solid phase anion exchange resins.

All pMACS vectors have been completely sequenced. pMACS vector sequences and maps are available on the website **www.miltenyibiotec.com**.

pMACS K^k.II plasmid
pMACS K^k.II is a 5.2 kb eukaryotic expression vector encoding a truncated mouse H-2K^k surface molecule. The surface molecule is expressed from the mouse H-2K^k promoter providing a high expression level in most mammalian cells.
pMACS K^k.II can be used for co-transfection in combination with an individual vector for gene-of-interest expression.
Additionally, pMACS K^k.II can be used for single-vector transfection after cloning the gene-of-interest into the multiple cloning site (MCS). The gene-of-interest is then expressed from the SV40 promoter/enhancer providing a high expression level in most mammalian cells[9]. The cloned sequence should include a Kozak sequence[†] and start and stop codons.

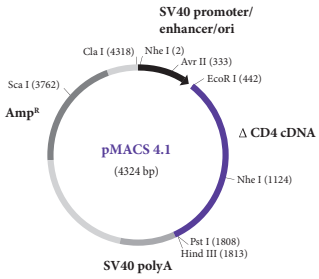


Multiple cloning site:

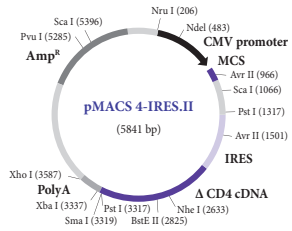
EcoRV	MluI	SacI	SmaI	
GAATTCGATATCACTAGTACGCGTCGACGAGCTCGGATCCCGGGAAGCTT				
CTTAAGCTATAGTGATCATGCGCAGCTGCTCGAGCCTAGGGCCCTTCGAA				
EcoRI	SpeI	SalI	BamHI	HindIII

Location of features		
MCS	Multiple Cloning Site	1-45
PolyA	SV40 early mRNA polyadenylation signal	105-288
Ori	ColE1 origin of replication	628-1300
Amp^R	β-lactamase open reading frame	1399-2259
PolyA	H-2K^k terminator and polyadenylation signal (reverse orientation)	2543-2378
Δ H-2K^k cDNA	H-2K^k open reading frame (cytoplasmatic region deleted, reverse orientation)	3563-2543
H-2K^k promoter	H-2K^k promoter (reverse orientation)	4700-3563
SV40 promoter	SV40 promoter/enhancer	4820-5122
	SV40 origin of replication	5035-5122

pMACS 4.1 plasmid
pMACS 4.1 is a 4.3 kb eukaryotic co-transfection vector for expression of the truncated, human CD4 surface molecule. The surface molecule is expressed from the SV40 promoter/enhancer providing a high expression level in most mammalian cells[9].



pMACS 4-IRES.II plasmid
pMACS 4-IRES.II is a 5.9 kb eukaryotic, bicistronic expression vector containing a multiple cloning site (MCS) followed by an IRES (internal ribosome entry site) element from EMCV* and the truncated CD4 cDNA. pMACS 4-IRES.II gene expression is driven from the human cytomegalovirus major immediate early promoter/enhancer (CMV promoter*). When a gene-of-interest is cloned into the MCS of pMACS 4-IRES.II, a bicistronic mRNA is transcribed which encodes the gene-of-interest as well as the truncated CD4 molecule. The IRES element enables the translation of two open reading frames from one mRNA, e.g., the gene-of-interest as well as the CD4 selection marker.
Transfected cells which express the gene-of-interest in high amounts are specifically enriched by using the pMACSelect 4-IRES.II vector.



Multiple cloning site

AgeI	BsiWI	EcoRV	NarI	SfiI	AscI
ACCGGTTAACGTACGTCTCAAGCTCCAGATATCGATTGGCGCCTTAATTAAGGCCTAGGCGGCCGCGGCGCGCCGAATTC					
TGGCCAATTGCATGCAGAGTTCGAGGTCTATAGCTAACCGCGGAATTAATTCCGGATCCGCCGGCGCCGGCGGCTTAAG					
HpaI	BsmBI	XcmI ClaI	PacI	NotI	BssHII	EcoRI

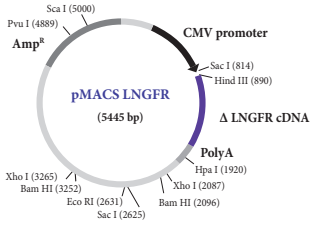
! **Note:** BsmB I cleaved vector is compatible with a Hind III restricted fragment.

! **Note:** Clone open-reading-frame of gene-of-interest with Kozak sequence[†] (see page 30) and start- and stop codons. Do not clone PolyA signal sequence into MCS.

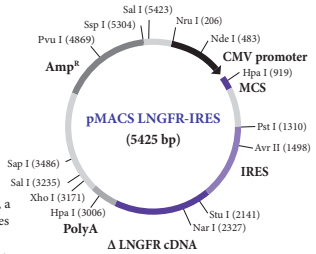
Location of features		
CMV promoter*	Cytomegalovirus IE promoter	232 - 820
MCS	Multiple cloning site	913 - 992
IRES	*Internal ribosome entry site of encephalomyocarditis virus (EMCV)	1348-1933
Δ CD4 cDNA	CD4 open reading frame (cytoplasmatic region deleted)	2031-3302
PolyA	Polyadenylation signal	3337-3615
Amp^R	β-lactamase open reading frame (reverse orientation)	5734-4877

* **Notice to purchaser:** Please see CMV promoter and EMCV IRES sequence legal statements.

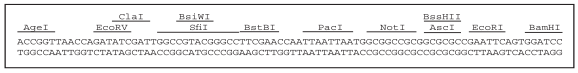
pMACS LNGFR
pMACS LNGFR is a 5.4 kb eukaryotic co-transfection vector for expression of the truncated, human, low-affinity nerve growth factor receptor (LNGFR). The surface molecule is expressed from the highly active human cytomegalovirus major immediate early promoter/enhancer (CMV promoter*).



pMACS LNGFR-IRES
pMACS LNGFR-IRES is a 5.4 kb eukaryotic, bicistronic expression vector containing a multiple cloning site (MCS) followed by an IRES (internal ribosome entry site) element from EMCV* and the truncated human, low-affinity nerve growth factor receptor (LNGFR) cDNA. pMACS LNGFR-IRES gene expression is driven from the human cytomegalovirus major immediate early promoter/enhancer (CMV promoter*). When a gene-of-interest is cloned into the MCS of pMACS LNGFR-IRES, a bicistronic mRNA is transcribed which encodes the gene-of-interest as well as the truncated LNGFR molecule. The IRES element enables the translation of two open reading frames from one mRNA, e.g., the gene-of-interest as well as the LNGFR selection marker. Transfected cells which express the gene-of-interest in high amounts are specifically enriched by using the pMACS LNGFR-IRES vector.



Multiple cloning site

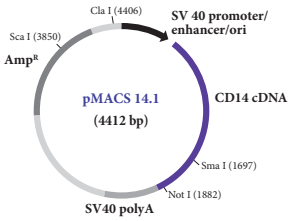


! **Note:** Clone open-reading-frame of gene-of-interest with Kozak sequence‡ (see page 30) and start- and stop codons. Do not clone PolyA signal sequence into MCS.

Location of features		
CMV promoter*	Cytomegalovirus IE promoter	232-820
MCS	Multiple cloning site	913-998
IVS	Synthetic intron	1030-1325
IRES*	Internal ribosome entry site of encephalomyecarditis virus (EMCV)	1351-1936
Δ LNGFR cDNA	LNGFR open reading frame	2040-2864
	(cytoplasmatic region deleted)	2040-2864
PolyA	Polyadenylation signal	2885-3108
Amp^R	β-lactamase open reading frame (reverse orientation)	5289-4429

* **Notice to purchaser:** Please see CMV promoter and EMCV IRES sequence legal statements.

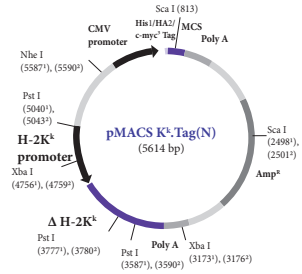
pMACS 14.1 control plasmid
The 4.4 kb control plasmid pMACS 14.1 encodes a human CD14 surface molecule. The surface molecule is expressed from the SV40 promoter/enhancer. The human CD14 molecule is anchored in the plasma membrane via a glycophosphoinositol linker (see also 2. "Set up of the MACSelect™ System"). The CD14 molecule encoded by pMACS 14.1 is not truncated and should not be used as a selection marker.

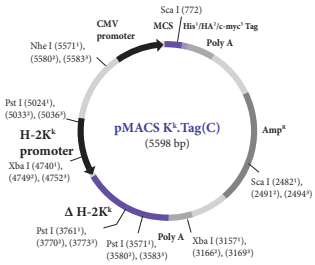


MACSelect K^k Tag Vector Sets
- -MACSelect K^k c-myc Vector Set
- -MACSelect K^k HA Vector Set
- -MACSelect K^k His Vector Set

The MACSelect K^k Tag Vector Set contains two pMACS K^k. Tag cloning vectors for eukaryotic expression of a tagged gene-of-interest and a MACSelect surface marker. pMACS K^k. Tag(N) contains a CMV promoter*, followed by the epitope tag sequence in front of the multiple cloning site (MCS) for insertion of the gene-of-interest (N-terminal tag). pMACS K^k. Tag (C) contains a CMV promoter*, followed by the MCS for insertion of the gene-of-interest in front of the epitope tag sequence (C-terminal tag). pMACS K^k. Tag vectors enable the expression of a tagged gene-of-interest, the enrichment of transfected cells with the MACSelect System, and the magnetic isolation of the tagged protein with a µMACS Tag Isolation Kit. The MACSelect marker ist the truncated, mouse H-2K^k cell surface receptor.

* **Notice to purchaser:** Please see CMV promoter legal statements.



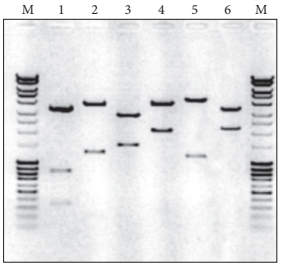


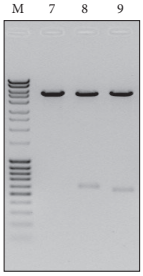
‡Kozak sequence:
The consensus sequence for initiation of translation in vertebrates
(also called Kozak sequence) is:

$$\frac{A}{G}CC\frac{A}{G}CC\mathbf{ATG}G$$

[1] pMACS K^k.His (N/C)
[2] pMACS K^k.HA (N/C)
[3] pMACS K^k.c-myc (N/C)

Diagnostic enzymatic restriction analysis of pMACS vectors
pMACS vectors can be distinguished by performing the enzymatic restrictions given in the table below. When separating the DNA after enzymatic plasmid restriction on an agarose gel, the DNA fragments, shown in the following pictures, are visible. For details see respective table.





Lane	MACSelect vector	Application	Restriction	Position	Fragments (kb)
1	pMACS 14.1 4412 bp	Cotransfection; control vector	Pst I	747 1.079 1.877	0.34 0.79 3.28
2	pMACS K^k.II 5229 bp	Cotransfection and cloning	Pst I	2.796 2.986 4.245	0.19 1.26 3.78
3	pMACS 4.1 4324 bp	Cotransfection	Avr II+Pst I	333 1.808	1.47 2.86
4	pMACS 4-IRES.II 5841 bp	Cloning	Pst I	1.317 3.317	2.00 3.84
5	pMACS LNGFR 5445 bp	Cotransfection	Xho I	2.087 3.265	1.18 4.27
6	pMACS LNGFR-IRES 5425 bp	Cloning	Hpa I	919 3.006	2.08 3.34
M	MassRuler™ DNA Ladder (MBI Fermentas)				

Lane	MACSelect vector	Application	Restriction	Position	Fragments (kb)
7	pMACS K^k.II 5229 bp	Cotransfection and cloning	Nde I + Eco RI	411	5.22
8	pMACS K^k.Tag (N) 5614 / 5617 bp	Cloning	Nde I + Eco RI	253 824 / 827	0.57 5.04
9	pMACS K^k.Tag (C) 5598 / 5607 / 5610 bp	Cloning	Nde I + Eco RI	253 783	0.53 5.07 / 5.08
M	MassRuler™ DNA Ladder (MBI Fermentas)				

7. References

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