MACS molecular

Time flies, and you are the pilot.

MACSelect™ System – the smart selection after each transfection

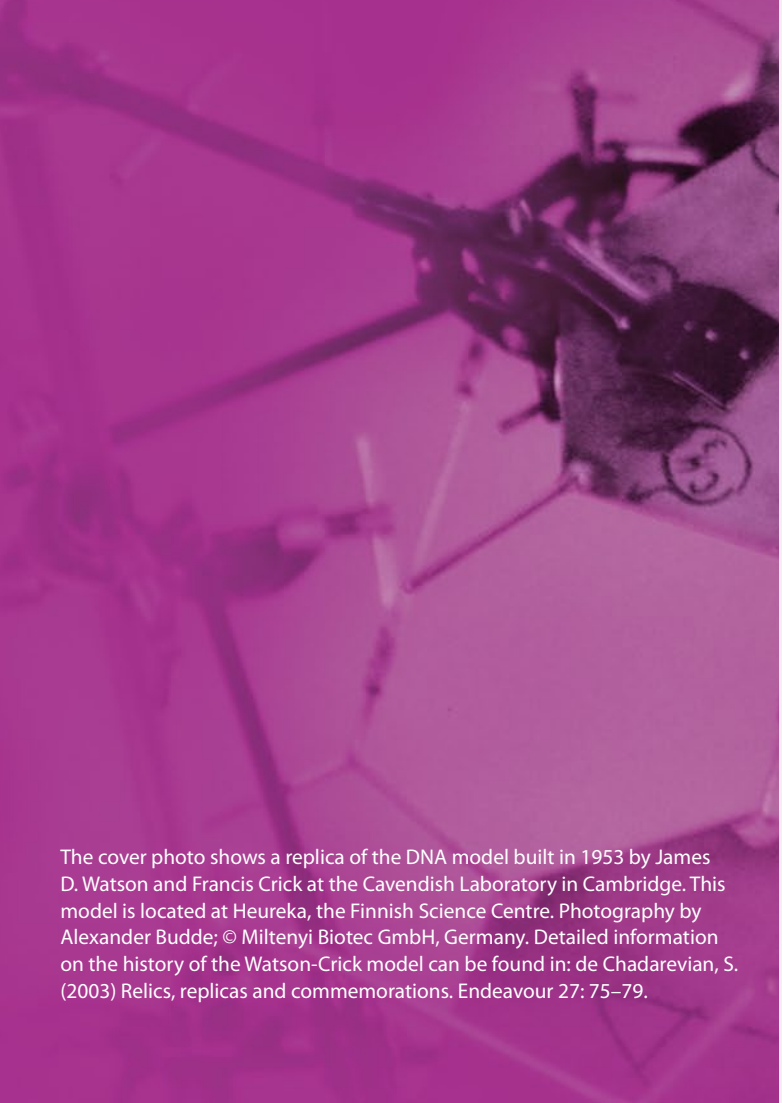
Minimal time, maximal results

Cell selection made easy

For any cell type

For any transfection method

3 hours only



The cover photo shows a replica of the DNA model built in 1953 by James D. Watson and Francis Crick at the Cavendish Laboratory in Cambridge. This model is located at Heureka, the Finnish Science Centre. Photography by Alexander Budde; © Miltenyi Biotec GmbH, Germany. Detailed information on the history of the Watson-Crick model can be found in: de Chadarevian, S. (2003) Relics, replicas and commemorations. Endeavour 27: 75–79.

Many positives, low background.
The MACSelect™ – Transfected Cell Selection System enables the magnetic enrichment of transfected mammalian cells. Primary cells or difficult-to-transfect cell lines, adherent or suspension cells can be selected after the regular transfection. The high percentage of positive cells and the minimized background of non-transfected cells increase significance and reliability of downstream analyses.

Selection in 3 hours only
Enrichment of transfected cells with MACSelect takes 3 hours versus several weeks for antibiotic treatment. Stable cell lines can be generated by repeated MACSelect isolations.

Gentle MACS® Technology
MACS® MicroBeads are ultrasmall (approx. 50 nm Ø), superparamagnetic, biodegradable, and non-toxic to cells. Thus, transfected cells are magnetically selected without affecting cell function or viability. Isolated cells can directly be used for functional studies or cell culture. Antibiotic treatment is no longer required.

No change of transfection method
MACSelect works with any transfection protocol: electroporation, calcium-phosphate, lipofection or nucleofection. Just add the MACSelect marker!

MACSelect is a smart system:

By co-expressing a marker on the cell surface, transfected cells can be magnetically labeled and separated from untransfected cells. Three different cell surface markers are available so that MACSelect is compatible with any cell type: human CD4, mouse MHC class I molecule H-2K^k, and human low-affinity nerve growth factor receptor (LNGFR). All pMACS vectors encode surface markers with truncated cytoplasmatic domains.

Simply clone your gene-of-interest into a pMACS vector or use a pMACS co-transfection vector. For transfection, any method can be used. A few hours later, transfected cells are labeled with MACSelect MicroBeads and separated on a MACS Column by magnetic force. This can be achieved either manually using MS or LS Columns and a MACS Separator, or – for larger throughput – automated with the autoMACS Separator.

Universal application for each transfection

- primary cells and cell lines
- adherent and suspension cells
- stable and transient transfections

and any throughput

- manual and automated cell selection

matching various research demands including

- functional gene analysis
- drug screening
- signal transduction studies
- reporter assays
- RNAi knockdown

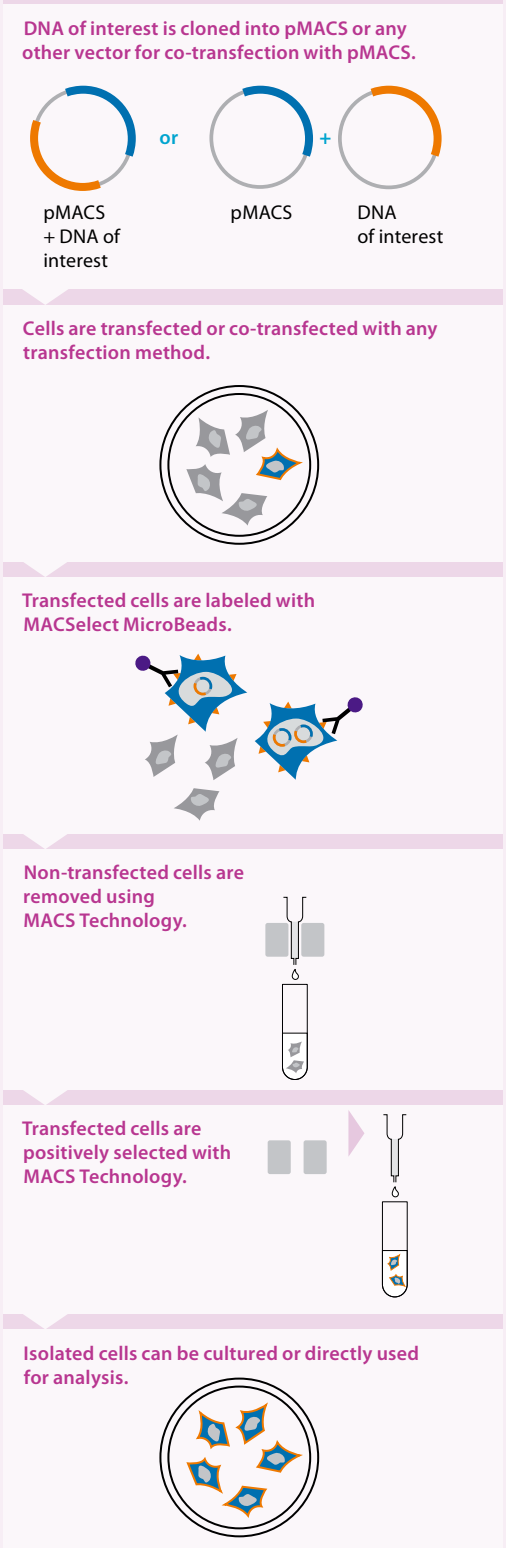


Figure 7: MACSelect procedure

Enrich transfected cells for sensitive analyses

1

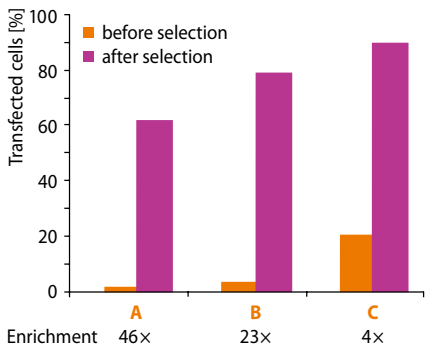


Figure 1: Three examples (A, B and C) for the enrichment of CHO cells which were co-transfected with pMACS Kk.II and pMACS 14.1 encoding CD14 as the gene-of-interest.

2

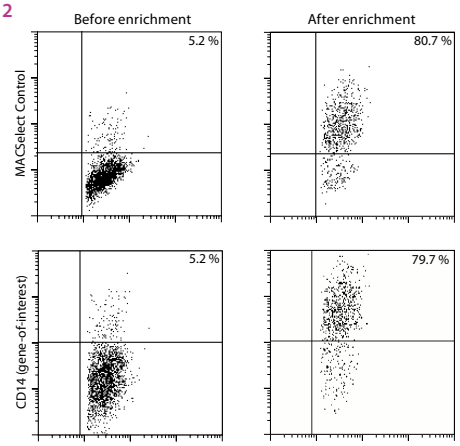
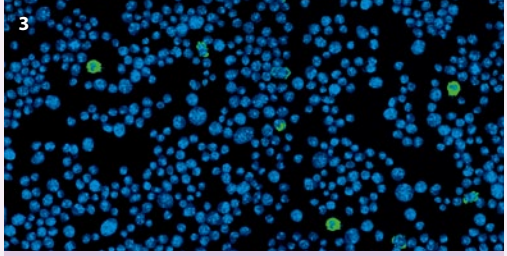


Figure 2: MACSelect enrichment of 1881 cells transfected with pMACS LNGFR-IRES containing CD14 as gene-of-interest. 18 h after transfection, cells were labeled with MACSelect LNGFR MicroBeads and (after removing an aliquot) separated using MS Columns. Cell aliquots were stained with MACSelect Control FITC antibody (top) or with CD14-PE antibody (bottom) before (left) and after (right) MACSelect enrichment. Percentage of positive cells increased from 5.2% (MACSelect Control and CD14 positive cells) to 80.7% (MACSelect Control positive cells) or 79,7% (CD14 positive cells) respectively.



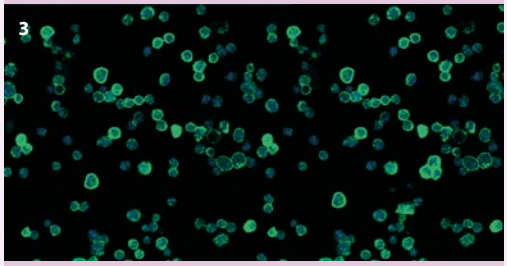


Figure 3: CHO cells were transfected with pMACS 4-IRES.II. Transfected cells were enriched using MACSelect 4 MicroBeads, fixed and stained with MACSelect Control FITC Antibody/CD4-FITC Antibody. Cell nuclei were counterstained with Toto3 (Molecular Probes). Top: before enrichment, bottom: after enrichment.

4 A)

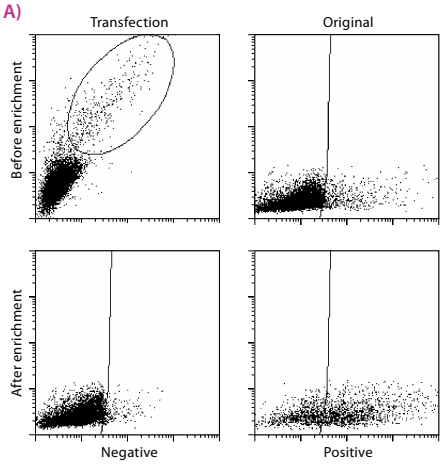
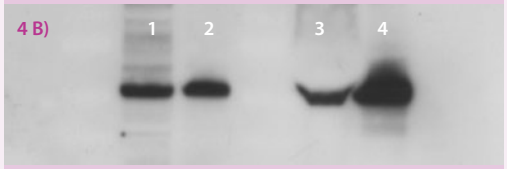


Figure 4: Raji cells were transfected with pMACS Kk.HA(C) containing CD4 as gene-of interest. A, MACSelect cell enrichment: CD4-PE / Anti-H-2K^k-FITC double-staining of transfected cells shows coexpression of gene-of-interest and MACSelect surface marker, respectively (transfection). Cells were selected using MACSelect K^k MicroBeads and stained with CD4-PE antibody to monitor enrichment. Percentage of gene-of-interest positive cells increased from 6.6% before MACSelect (origin) to 60.8% (positive); negative fraction (negative). B, CCD4-HA protein-of-interest was detected via Western blot: Lane 1,3: 10^5 cells were lysed before (origin, lane 1) and after (positive, lane 3) MACSelect enrichment; lane 2,4: CD4-HA was immunopurified from a lysate of 10^6 cells from the original (lane 2) or positive (lane 4) cell fraction using the µMACS HA Isolation Kit prior to loading onto the gel. CD4-HA was detected using CD4 antibody and Anti-IgG-HRP (B) or using Anti-HA-Biotin and Streptavidin-HRP (C).

4 B)



4 C)

The flexible MACSelect™ System

Choose the optimal surface marker for your cells: CD4, H-2K^k, or LNGFR

The MACSelect 4 System uses the truncated human CD4 molecule as a marker to select transfected cells. It can be used in virtually all CD4-negative cell lines. The truncated mouse MHC class I molecule H-2K^k is the selection marker of the MACSelect K^k System. H-2K^k expression is restricted to some rarely used mouse strains so that MACSelect K^k can be used in virtually any mammalian cell line. The MACSelect LNGFR System makes use of the truncated human low-affinity nerve growth factor receptor (LNGFR) molecule as a marker to select transfected cells. The LNGFR molecule is expressed in the central and peripheral nervous system, on bone marrow fibroblasts, follicular dendritic cells, and some mesenchymal cells.

Co-transfection or single vector transfection after cloning

For straightaway use of the MACSelect System, cells can be co-transfected with a pMACS vector and an expression vector containing the gene-of-interest. Alternatively, the gene-of-interest can be cloned into a pMACS vector for single vector transfection. Therefore regular (figure 6 B, C) and bicistronic (figure 6 A) vectors are available.

MACSelect Kits provide both alternatives – co-transfection or cloning. Additionally, MACSelect Tag Vector Sets streamline transfected cell enrichment and protein isolation: Each MACSelect Tag Vector Set includes two pMACS K^k vectors for N- or C-terminal epitope tagging with c-myc, His, or HA (figure 6 C). With a single cloning step, the gene-of-interest is introduced into a pMACS K^k.Tag vector, that encodes the H-2K^k surface marker for cell selection and the epitope-tagged target protein. Specific protein isolation can then be performed with µMACS™ Tag Protein Isolation Kits.

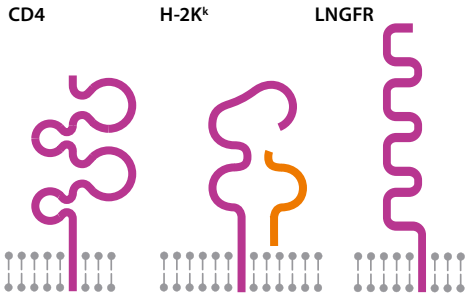
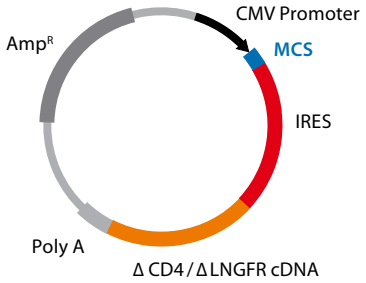
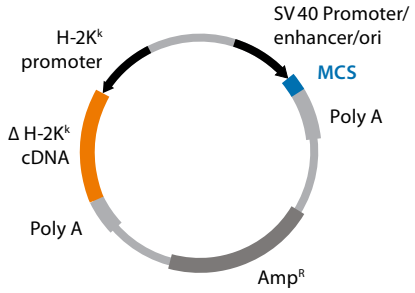


Figure 5: MACSelect surface markers
CD4 is naturally expressed on T helper cells, monocytes and dendritic cells. MACSelect 4 should not be used for such cell types of human origin. CD4 is trypsin sensitive.
H-2K^k expression is restricted to some rarely used mouse strains like AKR/J and CBA/Ca. MACSelect K^k should not be used for such murine cell types. H-2K^k requires co-expression of β-2-microglobulin.
LNGFR is expressed in the CNS and PNS on autonomic and sensory neurons and glial cells, on bone marrow fibroblasts, follicular dendritic cells, and some mesenchymal cells. MACSelect LNGFR should not be used for such cell types of human origin.

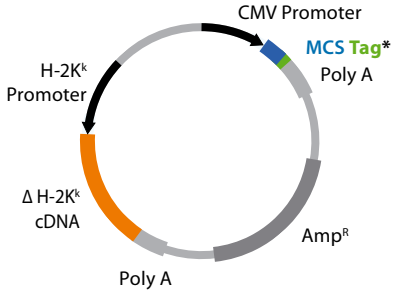
6 A) pMACS 4-IRES.II vector
pMACS LNGFR-IRES vector



6 B) pMACS K^k.II



6 C) pMACS K^k.Tag(C/N)



* C-or N-terminal HA-, His-, or c-myc-tag

Figure 6: pMACS vector maps

MACSelect™ – Transfected Cell Selection Kits			Order no.
MACSelect 4 – Transfected Cell Selection Kit	for 25 separations		130-091-988
MACSelect 4 MicroBeads	for 25 separations		130-070-101
MACSelect K^k – Transfected Cell Selection Kit	for 25 separations		130-091-986
MACSelect K^k MicroBeads	for 25 separations		130-070-201
MACSelect LNGFR – Transfected Cell Selection Kit	for 25 separations	NEW	130-091-879
MACSelect LNGFR MicroBeads	for 25 separations	NEW	130-091-330

Kit components: 2 mL MACSelect MicroBeads, pMACS cloning and co-transfection vector(s), control vector, (25 µg each), 3 Fluorochrome-conjugated antibodies incl. MACSelect Control FITC

MACSelect™ Vectors and Tag Vector Sets			Order no.
pMACS LNGFR	25 µg plasmid	NEW	130-091-890
pMACS LNGFR-IRES	25 µg plasmid	NEW	130-091-887
pMACS K^k.II	25 µg plasmid	NEW	130-091-889
pMACS 4.1	25 µg plasmid	NEW	130-091-886
pMACS 4-IRES.II	25 µg plasmid	NEW	130-091-888
MACSelect K^k c-myc Vector Set	2×25 µg plasmid	NEW	130-092-085
MACSelect K^k HA Vector Set	2×25 µg plasmid	NEW	130-092-084
MACSelect K^k His Vector Set	2×25 µg plasmid	NEW	130-092-083

MACSelect™ Antibodies and accessories		Order no.
CD4-FITC, human	for 100 tests with up to 10^7 cells [1]	130-080-501
Anti-H-2K^k-FITC, mouse	for 100 tests with up to 10^7 cells [1]	130-085-101
CD 271 (LNGFR)-FITC	for 100 tests with up to 10^7 cells [1]	130-091-917
MACSelect Control FITC Antibody	for 100 tests with up to 10^7 cells [1]	130-090-326
CD14-FITC	for 100 tests with up to 10^7 cells [1]	130-080-701
Dead Cell Removal Kit	10^9 total cells	130-090-101

[1]One test corresponds to fluorescent labeling of up to 10^7 cells in a total volume of 100 µL

Isolation of epitope tagged proteins and their binding partners		Order no.
µMACS HA Isolation Kit	40 rxns	130-091-122
µMACS c-myc Isolation Kit	40 rxns	130-091-123
µMACS His Isolation Kit	40 rxns	130-091-124

Kit components: 2 mL µMACS MicroBeads, Lysis Buffer, Wash Buffer 1, Wash Buffer 2, Elution Buffer

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