Miltenyi Biotec

MACSelect™ K^k His Vector Set

Order no. 130-092-083

Contents

1. Description
   - 1.1 Principle of MACSelect™ transfected cell separation
   - 1.2 Background and product applications
   - 1.3 Reagent and instrument requirements
   - 1.4 Vector map
   - 1.5 Location of features
   - 1.6 Plasmid sequence
   - 1.7 Multiple cloning site
2. Protocol
   - 2.1 Plasmid preparation
   - 2.2 Cloning of the gene-of-interest
   - 2.3 MACSelect™ enrichment of transfected cells

1. Description

This product is for research use only.

Components	**25 µg pMACS K^k.His(N) plasmid** (lyophilized) **25 µg pMACS K^k.His(C) plasmid** (lyophilized)
Capacity	2×25 µg plasmid DNA.
Storage	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 Principle of MACSelect™ transfected cell separation

The MACSelect System enables the enrichment of transfected cells. For enrichment, cells are transfected with the gene-of-interest and a cell surface marker encoded by the respective pMACS vector. After cell transfection with any common method, only the transfected cells express the surface marker and are labeled and magnetically enriched with MACSelect MicroBeads, a MACS® Separator, and MACS Separation Columns.

1.2 Background and product applications

The MACSelect K^k His Vector Set contains two pMACS K^k.His cloning vectors for eukaryotic expression of an His-tagged gene-of-interest and a MACSelect surface marker. pMACS K^k.His(N) contains a CMV promoter, followed by the His epitope tag sequence in front of the multiple cloning site (MCS) for insertion of the gene-of-interest (N-terminal His tag). pMACS K^k.His(C) contains a CMV promoter, followed by the MCS for insertion of the gene-of-interest in front of the His epitope tag sequence (C-terminal His tag). pMACS K^k.His vectors enable the expression of an His-tagged gene-of-interest, the enrichment of transfected cells with the MACSelect System, and the magnetic isolation of the His-tagged protein with the µMACS™ His Isolation Kit (# 130-091-124). The MACSelect marker is the truncated, mouse H-$2K^k$ cell surface receptor. By using a vector with an H-$2K^k$ encoding gene with a truncated cytoplasmic domain, signal transduction by the H-$2K^k$ protein is not possible. The epitope recognized by MACSelect K^k MicroBeads is not sensitive to trypsin digestion.

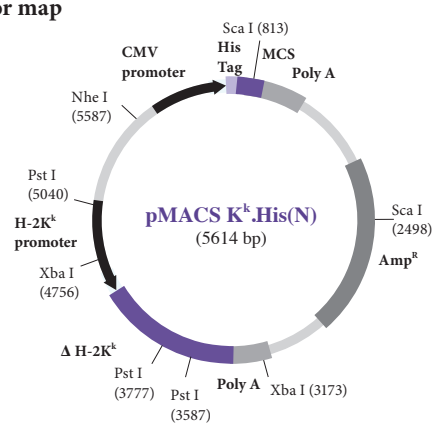
For further information on MACSelect - Transfected Cell Selection please refer to the MACSelect User manual.

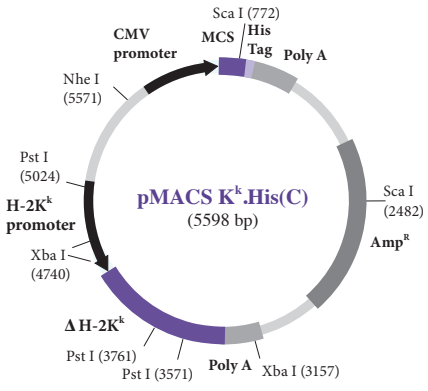
▲ **Note:** H-$2K^k$ expression is restricted to some rarely used mouse strains like AKR/J and CBA/Ca. The MACSelect K^k System is not suitable for these murine cell types.

1.3 Reagent and instrument requirements

- Sterile, distilled water
- Reagents for transformation and purification of plasmid DNA (e.g. commonly used *E. coli* strains such as Top10, DEAE solid phase anion exchange resins).
- Reagents for vector cloning.
- For transfected cell enrichment: please refer to the MACSelect User manual.
- For His-tagged protein isolation: µMACS His Protein Isolation Kit (# 130-091-124).

1.4 Vector map





Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-001-619.03

1.5 Location of features

pMACS K^k.His(N); 5614 bp

pCMV	Cytomegalovirus IE promoter	1–589
pT7	T7 promoter	633–651
His Tag	6His tag (HHHHHH)	705–722
MCS	Multiple cloning site	723–834
polyA	SV40 polyadenylation signal	840–1084
colE1	ColE1 origin of replication	1423–2095
Amp^R	β-lactamase ORF	2194–3054
K^k-PolyA	H-2K^k polyadenylation signal (reverse orientation)	3338–3173
ΔH-2K^k	truncated H-2K^k ORF (reverse orientation)	4358–3339
pH-2K^k	H-2K^k promoter (reverse orientation)	5499–4367

pMACS K^k.His(C); 5598 bp

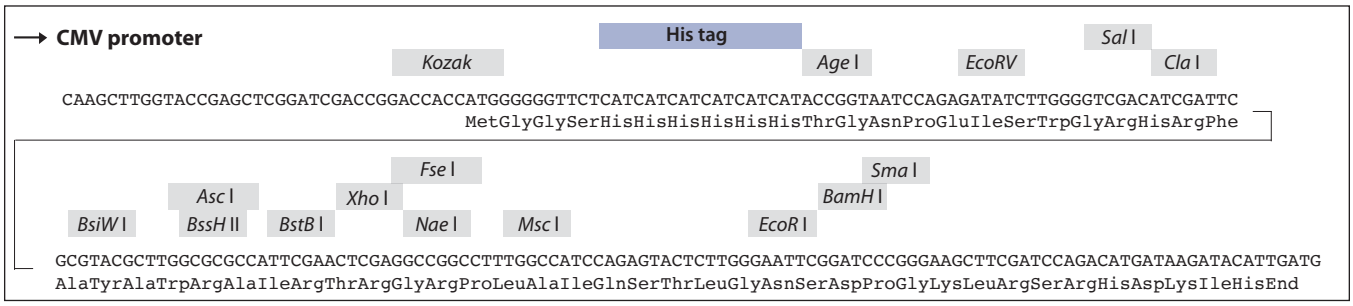
pCMV	Cytomegalovirus IE promoter	1–589
pT7	T7 promoter	633–651
MCS	Multiple cloning site	682–789
His Tag	6His tag (HHHHHH)	790–807
polyA	SV40 polyadenylation signal	824–1068
colE1	ColE1 origin of replication	1407–2079
Amp^R	β-lactamase ORF	2178–3038
K^k-PolyA	H-2K^k polyadenylation signal (reverse orientation)	3322–3157
ΔH-2K^k	truncated H-2K^k ORF (reverse orientation)	4342–3323
pH-2K^k	H-2K^k promoter (reverse orientation)	5483–4351

1.6 Plasmid sequence

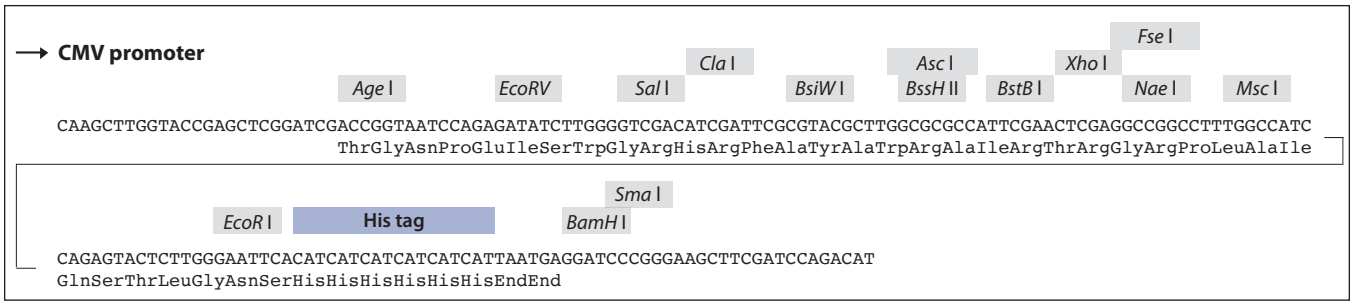
The plasmid sequence can be downloaded from **www.miltenyibiotec.com** (use search word "MACSelect").

1.7 Multiple cloning site

pMACSK^k.His(N)



pMACSK^k.His(C)



▲ Clone open-reading-frame of gene-of-interest with Kozak sequence‡ and start- codon for pMACS K^k.His(C). A stop codon is required when cloning into pMACS K^k.His(N).

2. Protocol

2.1 Plasmid preparation

pMACS K^k.His 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. DEAE solid phase anion exchange resins.

2.2 Cloning of the gene-of-interest

For expression of a His tagged protein, please make sure to clone the gene-of-interest into the correct reading frame. Please refer to **www.miltenyibiotec.com** (use search word "MACSelect") for further information.

2.3 MACSelect™ enrichment of transfected cells

Please refer to MACSelect User manual (MACSelect User manual is included in MACSelect Kits and is available at **www.miltenyibiotec.com**).

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

*The CMV promoter is covered under US patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a licence from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

‡Kozak sequence:
The consensus sequence for initiation of translation in vertebrates (also called Kozak sequence) is: $\frac{A}{G}$CC$\frac{A}{G}$CC**ATG**G

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

140-001-619.03

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

MACS, MACSelect, the Miltenyi Biotec logo, and µMACS are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.

Copyright © 2021 Miltenyi Biotec and/or its affiliates. All rights reserved.