Miltenyi Biotec

MACSelect™ K^k HA Vector Set

Order no. 130-092-084

Contents

1. Description

This product is for research use only.

Components	**25 µg pMACS K^k.HA(N) plasmid** (lyophilized) **25 µg pMACS K^k.HA(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 HA Vector Set contains two pMACS K^k.HA cloning vectors for eukaryotic expression of an HA tagged gene-of-interest and a MACSelect surface marker. pMACS K^k.HA(N) contains a CMV promoter, followed by the HA epitope tag sequence in front of the multiple cloning site (MCS) for insertion of the gene-of-interest (N-terminal HA tag). pMACS K^k.HA(C) contains a CMV promoter, followed by the MCS for insertion of the gene-of-interest in front of the HA epitope tag sequence (C-terminal HA tag). pMACS K^k.HA vectors enable the expression of an HA-tagged gene-of-interest, the enrichment of transfected cells with the MACSelect System, and the magnetic isolation of the HA-tagged protein with the µMACS™ HA Isolation Kit (# 130-091-122). 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..

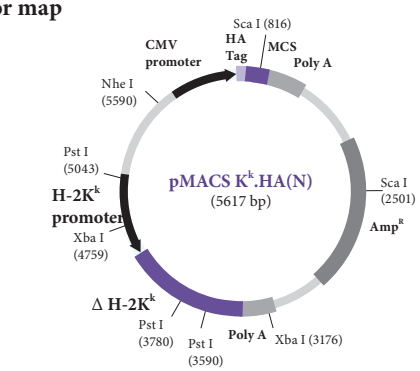
For further information on MACSelect - Transfected Cell Selection please refer to the MACSelect User manual.

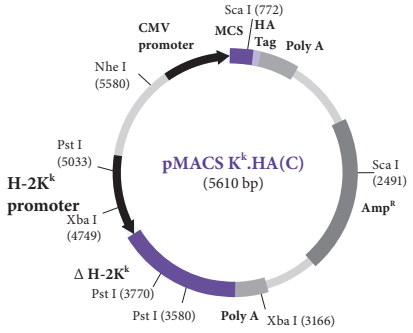
▲ **Note:** H-2K^k expression is restricted to some rarely used mouse strains like AKR/J and CBA/Ca. The MACSelect K^k System is not suitable for these murine cell types.

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 HA tagged protein isolation: µMACS HA Protein Isolation Kit (# 130-091-122).
- For HA tagged protein detection: Anti-HA-HRP (# 130-091-972).
- For immunofluorescent detection of HA tagged protein: Anti-HA-FITC (# 130-092-256), Anti-HA-PE (# 130-092-257), or Anti-HA-Biotin (# 130-092-258)..

1.4 Vector map





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140-001-586.03

1.5 Location of features

pMACS K^k.HA(N); 5617 bp

pCMV	Cytomegalovirus IE promoter	1–589
pT7	T7 promoter	633–651
HA	Hemagglutinin HA tag (YPYDVPDYA)	699–725
MCS	Multiple cloning site	726–837
polyA	SV40 polyadenylation signal	843–1087
colE1	ColE1 origin of replication	1426–2098
Amp^R	β-lactamase ORF	2197–3057
K^k-PolyA	H-2K^k polyadenylation signal (reverse orientation)	3341–3176
ΔH-2K^k	truncated H-2K^k ORF (reverse orientation)	4361–3342
pH-2K^k	H-2K^k promoter (reverse orientation)	5502–4370

pMACS K^k.HA(C); 5607 bp

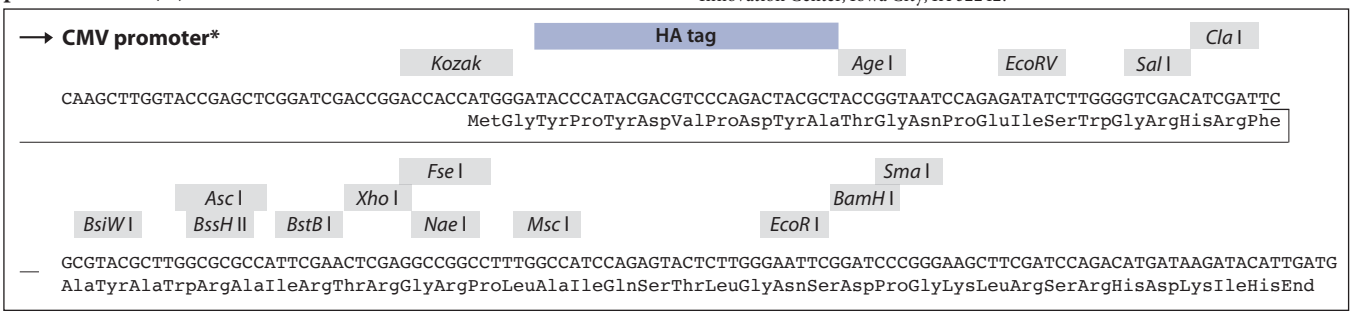
pCMV	Cytomegalovirus IE promoter	1–589
pT7	T7 promoter	633–651
MCS	Multiple cloning site	682–789
HA	Hemagglutinin HA tag (YPYDVPDYA)	790–816
polyA	SV40 polyadenylation signal	833–1077
colE1	ColE1 origin of replication	1416–2088
Amp^R	β-lactamase ORF	2187–3047
K^k-PolyA	H-2K^k polyadenylation signal (reverse orientation)	3331–3166
ΔH-2K^k	truncated H-2K^k ORF (reverse orientation)	4351–3332
pH-2K^k	H-2K^k promoter (reverse orientation)	5492–4360

1.6 Plasmid sequence

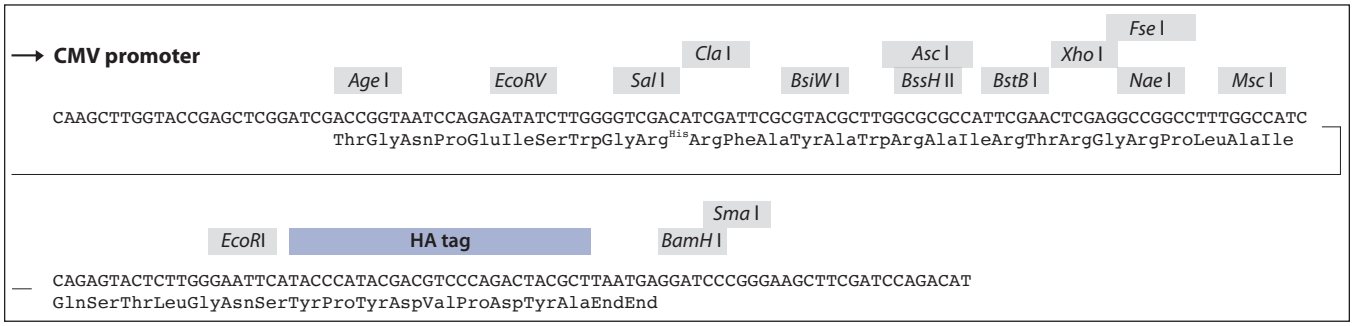
The plasmid sequence can be downloaded from **www.miltenyibiotec.com** (use search word "MACSelect").

1.7 Multiple cloning site

pMACSK^k.HA(N)



pMACSK^k.HA(C)



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

2. Protocol

2.1 Plasmid preparation

pMACS K^k.HA 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 HA 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

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