MACS

Starting protocols
molecular biology

µMACS Streptavidin Kit

Tips & hints

µMACS Streptavidin Kit Order No. 130-074-101

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Tips & hints: Purification of biotinylated molecules and their targets by using the µMACS Streptavidin Kit

For the general procedure plese refer to the data sheet of the **µMACS Streptavidin Kit**. To optimize your experimental set-up or for troubleshooting, please refer to these general checkpoints and recommendations.

I. General checkpoints

DNA probes

- **Purity:** The synthesized biotinylated DNA oligonucleotide should be purified by HPLC. HPLC purified probes avoid unspecific binding.
- **Length:** The DNA probe should be long enough. Too short probes fail either to bind to other nucleic acids or to catch DNA binding proteins.

  For specific binding of nucleic acids, the single stranded DNA probe should be at least 25-30 bases long.

  For the isolation of DNA binding proteins, the double stranded DNA probe should be at least 30-35 base pairs long, and the protein binding site should be placed at least 20 base pairs away from the biotin residue.

  If two biotins on opposite ends are used, add a further 5 bases to the DNA (i.e. 30-35 bases for single stranded DNA and 35-40 base pairs for double stranded DNA).

Labeling

- **Dilution:**

  The Streptavidin MicroBeads in the binding reaction work best when diluted between 1:2 and 1:10.

  They should not be diluted more than 1:10.
- **Capacity:**

  For complete binding of biotinylated molecules, use enough MicroBeads, if possible in excess.

  100 µl of Streptavidin MicroBeads bind up to 100 pmol of a biotinylated oligonucleotide.

  For long DNA with a size over a few hundred base pairs or if many biotin residues per molecule are available, the capacity may be significantly lower. As a rule of thumb, suppose that 20 pmol of these molecules can be bound.

  For a 5000 bp plasmid with a low number of biotin residues (< 5), at least 5 µg can be bound by 100 µl of Streptavidin MicroBeads. For a higher binding capacity, more MicroBeads should be added.
- **Biotinylation:**

  If you biotinylate the molecule to be used as a probe yourself, avoid hyperbiotinylation: < 5 biotin residues per molecule work best. A protocol for plasmid biotinylation is available from Miltenyi Biotec.

Column

Make sure the correct **Equilibration Buffer** is used. The buffer for protein applications is compatible with nucleic acids, but the buffer for nucleic acid applications is NOT appropriate for protein applications.

Washing

Ensure that the **composition of the washing buffer(s)** is compatible with the application:

- **salt concentration** – too high salt concentrations lead to disruption of protein binding, and to lower specificities in nucleic acid hybridization, respectively; too low salt concentrations lead to protein background, and may interfere with nucleic acid hybridization.
- **pH** – the pH should be optimal for the molecule of interest, e.g. pH 7.5-8 for DNA.
- **temperature** – to reduce unspecific hybridization, the temperature of the washing buffer may be increased for nucleic acids. Proteins may be denatured or inactivated by elevated temperatures.

Elution

- **Composition** of the elution buffer: Generally, proteins are eluted with high salt buffers, while nucleic acids are eluted with low salt buffers, TE or water (DNA: pH 7.5-8.0, RNA: pH 5-5.5).
- **Temperature** of the elution buffer: To denaturate nucleic acids for elution, buffer temperature must be 80°C. Proteins are eluted at room temperature.
- Column **inside/outside magnet:** In most cases, the elution of the molecule of interest will take place with the column in the magnet. However, if the whole complex of µMACS Streptavidin MicroBeads and bound molecules should be eluted, the elution proceeds with the column outside the magnet; in this case, no special elution buffer is needed.

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All buffers

Ensure that **specific requirements** for the molecule of interest are taken into account, e.g.

- cofactors for the binding of proteins
- blocking reagents to prevent unspecific background
- inhibitors to reduce degradation or dephosphorylation.

Analysis by agarose gel electrophoresis

- For the analysis of nucleic acids by agarose gel electrophoresis, it is important to know that nucleic acids bound to Streptavidin MicroBeads do not migrate into the gel, but stick in the slot. Therefore, it is necessary to release the nucleic acids from the MicroBeads either by restriction enzymes, or by denaturating double stranded nucleic acids to get single stranded molecules from which the unbiotinylated molecules migrate into the gel.

2. How to optimize your experimental set-up, when . . .*

2.1. . . you find your molecule of interest in the flow-through of the column:

Possible reason	Solution
• too much DNA probe/too few MicroBeads used	• reduce the amount of DNA probe or increase the amount of MicroBeads so that the biotinylated probe can be completely captured by MicroBeads
• low quality of DNA probe	• purify the DNA probe by HPLC; check the length of the probe
• dilution of MicroBeads too high	• do not dilute the Beads more than 1:10
• annealing temperature too high	• check the correct annealing temperature
• wrong salt concentration in the binding reaction	• adjust salt concentration in the binding reaction (reduce for protein binding, increase for nucleic acid hybridization)
• proteins may require cofactors for binding	• add the appropriate cofactors to the binding reaction (e.g. Mg^{2+})
• too stringent conditions for protein-protein interactions	• check the requirements for a stable protein-protein binding (e.g. reduce salt concentration of the binding reaction)
• too much unspecific binding of other molecules	• add blocking reagents to the binding reaction (e.g. tRNA, poly[d(I:C)] for nucleic acids, BSA for proteins)
• too many biotin residues on the molecule (nucleic acid, protein)	• reduce the amount of biotin for the biotinylation of molecules (optimal: < 5 biotin residues per molecule)

2.2. . . you find your molecule of interest in the wash fraction:

Possible reason	Solution
• the salt concentration of the washing buffer is wrong	• adjust salt concentration in the washing buffer(s) (reduce for protein binding, increase for nucleic acid hybridization)
• proteins may require cofactors for binding	• add the appropriate cofactors to the washing buffer(s) (e.g. Mg^{2+})
• too stringent conditions for protein-protein interactions	• check the requirements for a stable protein-protein binding (e.g. reduce salt concentration of the washing buffer(s))

*You can find a glossary at the end of the document.

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2.3 . . . you find too many unspecific molecules in the eluate:

Possible reason	Solution
• washing steps not stringent enough	• increase stringency of the washing buffers(s): **for nucleic acids** by decreasing the salt concentration and/or increasing the temperature of the washing buffer(s) or adding of blocking reagents to the binding reaction, i.e. tRNA, poly[d(I:C)] **for proteins** by increasing the salt concentration or the addition of detergents (e.g. 0.1 % Tween 20) may help; also adding of blocking reagents to the binding reaction (BSA)
• unspecific DNA probe	• check for a specific DNA probe

2.4 . . . you do not find your target molecule in the eluate, the flow-through or the wash fractions:

Possible reason	Solution
• the molecule was degraded	• add specific inhibitors to all buffers (proteins: protease inhibitors like PMSF, Leupeptin; DNA: EDTA; RNA: Rnase inhibitors like Rnasin; phosphorylated proteins: phosphatase inhibitors like sodium orthovanadate, sodium fluoride)
• wrong elution buffer	• check the composition of the elution buffer; proteins are generally eluted by high salt conditions, whereas nucleic acids are eluted with low salt or in water
• elution buffer too cold (nucleic acids)	• use 80°C hot elution buffer (to denaturate double stranded nucleic acids)
• low quality of cell lysate or extract (with too less amounts of target molecule)	• check the lysate for the molecule of interest (e.g. by western blotting)
• inadequate analysis method	• check fractions by PCR (for nucleic acids) or SDS-PAGE, followed by silver staining or western blotting (for proteins)
• biotinylated probe is inadequate	• use a specific probe
• insufficient biotinylation of the probe molecule	• check biotinylation state

2.5 . . . the isolated protein is not native / functional:

Possible reason	Solution
• wrong equilibration buffer	• use the equilibration buffer for nucleic acids and the equilibration buffer for proteins appropriately; the equilibration buffer for nucleic acids is NOT compatible with proteins
• incompatible binding / washing / elution buffer(s)	• check the composition of all buffers and remove reagents which may destroy proteins (e.g. SDS)
• hot elution buffer	• use cold elution buffer (room temperature)

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3. Glossary

Binding reaction: Reaction in which the binding process occurs; it consists of Streptavidin MicroBeads, biotinylated probe and sample including the target molecule. In some reactions, the biotinylated probe and the target molecule may be identical (e.g. biotinylated cell surface receptors, which should be isolated).

Flow-through: The binding reaction is applied onto the column, and the part of it which is not retained by the column is called flow-through.

Wash fraction(s): Molecules which are retained on the column are washed with buffer(s) to reduce the background of unspecifically bound molecules. These fractions are called wash fraction(s). All of these should be collected and analyzed. It is useful to test wash buffers with different stringencies to determine the optimal purification strategy for the target molecule.

Elution fraction / eluate: To isolate the molecule of interest without the biotinylated probe, an appropriate elution buffer is applied to the column, leading to a release of the molecule of interest, while retaining the biotinylated probe and the MicroBeads. The resulting fraction containing the isolated target molecule is the eluate or elution fraction. The composition of the elution buffer depends on the application and the target molecule.
If the whole molecule(s)-MicroBead-complex is to be eluted, then the column must be removed from the magnet. In this case, the elution of the complex can be done with any buffer which fits into possible downstream applications.

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