MACS molecular

Phage display

µMACS Streptavidin Kit Order No. 130-074-101

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1. Background

The display of foreign peptides and proteins on the surface of filamentous bacteriophages, "phage display", has become a cornerstone of techniques to investigate molecular interactions.
Phage display is used:

- to identify and analyze features of surface proteins that interact with other proteins.
- to isolate new ligand that bind to particular amino acid sequences.
- to improve the affinity and specificity of the interaction between ligands and their target structures.

This protocol describes the principal procedure for phage display by using **µMACS Streptavidin MicroBeads**. A biotinylated target (e.g. protein) is incubated with the library and then "panned" with µMACS Streptavidin MicroBeads. Non-binding and unspecific phages are displaced by a pre-incubation step and stringent washing steps on the MACS Column, and either the phage or the phage-target-MicroBead complex is eluted.

1.1 Reagent and instrument requirements

- **Buffers:**
  Tris-L Buffer: 10 mM Tris/HCl pH 7.5
  Tris-H Buffer: 100 mM Tris/HCl pH 7.5
  TBST Buffer: 50 mM Tris pH 7.5, 150 mM NaCl, 0.1 % (v/v) Tween-20
  Elution Buffer N – nonspecific: 0.2 M Glycine-HCl pH 2.2, 1 mg/ml BSA
  or
  Elution Buffer S – specific: 50 mM Tris pH 7.5, 150 mM NaCl, 0.1–1 mM of a known ligand for the target OR ~100 µg/ml of the free target
- **µMACS Separator** (# 130-042-602) and **MultiStand** (# 130-042-303)
- **µ Columns** (# 130-042-701)
- (Optional) **MACSmix** (# 130-090-753)
- **µMACS Streptavidin Kit** (# 130-074-101)

2. Protocol

2.1 Biotinylation of the target molecule

Kits for biotinylation are commercially available. The biotinylation reaction must take place in a buffer that does not contain free amine groups, such as $NaHCO_3$, pH 8.6 (i.e. do not use TRIS). The degree of biotinylation should be in the range of 2-3 biotin residues per target molecule. This can be quantitated by the HABA assay (Pierce). Free biotin has to be removed by gel filtration, dialysis, or ultrafiltration (Centricon).

2.2 Removal of unspecific phages

1. Combine 10 µl of **Tris-H Buffer**, 100 µl of **µMACS Streptavidin MicroBeads** and about 10^{11} pfu (plaque forming units) of the input phage in a tube. For best results, the dilution of the µMACS Streptavidin MicroBeads should be no more than 1:10.
   ▲ **Note:** Do not add biotinylated target at this point.
2. Incubate for 5 minutes.
3. Place a µ Column in the magnetic field of the µMACS Separator. Prepare the column by applying 100 µl of **Equilibration Buffer for protein applications** (supplied with the kit) on top of the column. Wash with 2x200 µl of **Tris-L Buffer**.
4. Apply the phage-MicroBeads mix onto the µ Column and let it run through. Phages that unspecifically bind to µMACS Streptavidin MicroBeads are retained on the column.
5. Collect the flow-through in a tube.
6. Add 50 µl of **Tris-L Buffer** to the column, and collect the flow-through in the same tube. Discard the column.

2.3 Binding of the biotinylated target to the phages

1. Mix the collected phages (= the collected flow-through from the µ Column) with about 50 pmol of the biotinylated target molecule. Metal ions, cofactors, etc. may be added if necessary to stabilize the target.
2. Incubate for 15 minutes at room temperature. To accelerate the binding kinetics, you may use the **MACSmix** tube rotator.
   ▲ **Note:** The amount of salt influences the binding reaction. The addition of salt leads to a lower specificity between target-phage complexes.

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Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925

2.4 Magnetic labeling

Magnetic labeling of the target-phage complex

1. Add 100 µl of **µMACS Streptavidin MicroBeads** to the target-phage solution. For best results, the dilution of the µMACS Streptavidin Microbeads should be no more than 1:10.
2. Mix and incubate for 10 minutes at room temperature.

2.5 Magnetic separation

1. Place a second µ Column in the magnetic field of the µMACS Separator. Prepare the column by applying 100 µl of **Equilibration Buffer for protein applications** (supplied with the kit) on top of the column. Wash with 2 x 200 µl of Tris-L Buffer.
2. Apply the mixture containing magnetically labeled target-phage-MicroBead complexes on top of the second µ Column and let it pass through. The magnetically labeled complexes are retained on the column.
3. Wash with 10 x 200 µl of **TBST** to remove unbound phages.

   ▲ **Note:** The composition of the Buffer for washing may be adjusted (e.g. salt concentration) to achieve optimum conditions; higher salt concentrations lead to more stringent conditions. To displace any residual Streptavidin binding phages, a wash with 0.1 mM free biotin in TBST Buffer may be carried out.

Elution within the magnet

Elute bound phage with an appropriate **Elution Buffer** for the interaction being studied:

1. Use **Elution Buffer N** for a nonspecific disruption of binding. Pipette 20 µl of the Elution Buffer N on top of the column. Incubate for 5 minutes at room temperature. Add an additional 50-200 µl of Elution Buffer N, and collect the drops. Immediately neutralize with 1 M Tris-HCl (pH 9.0) and vortex.
2. Alternatively, **Elution Buffer S** may be used if either a known ligand for the target, or the free target to compete the bound phage away from the immobilized target on the MicroBeads is available. Pipette 20 µl of Elution Buffer S on top of the column matrix. Incubate for 10-30 minutes at room temperature. Add an additional 50-200 µl of the Elution Buffer S, and collect the drops.

Elution outside the magnet

This is recommended if the entire complex of biotinylated target, phage, and µMACS Streptavidin MicroBeads should be eluted.

1. Remove the column from the magnet.
2. Pipette 150-200 µl of an appropriate elution buffer (e.g. TRIS-L Buffer) on top of the column. Avoid contents in the elution buffer which may destroy the complex (e.g. SDS, high salt, extreme high or low pH).
3. The flow-through will contain the complex of biotinylated target, phage, and Streptavidin MicroBeads.