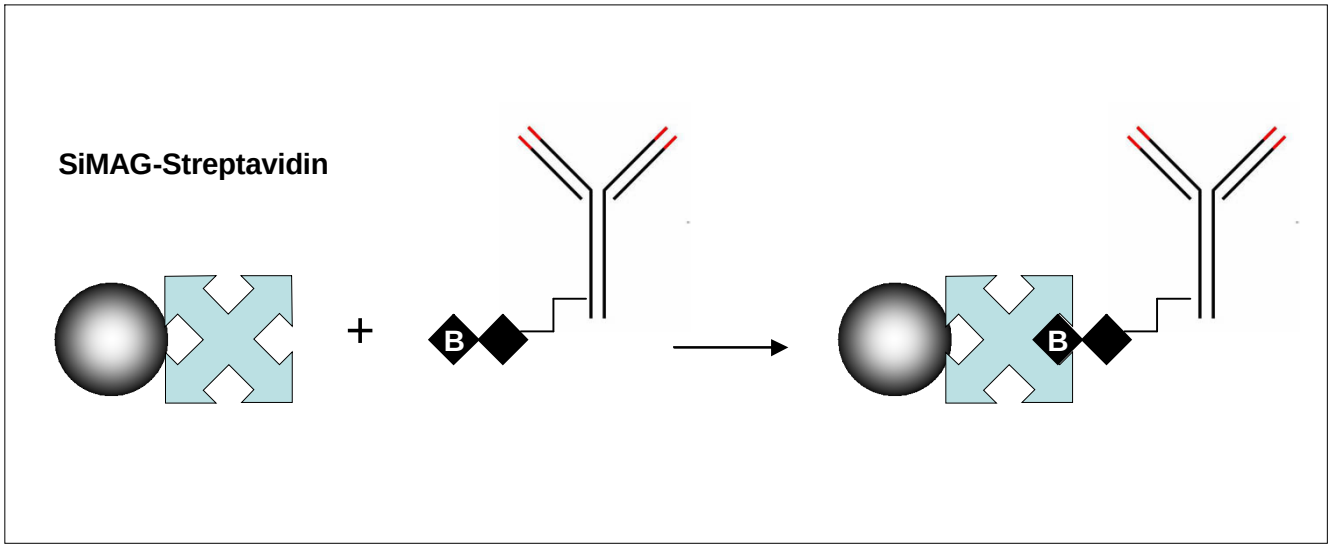
Immobilization of biotinylated ligands with SiMAG-Streptavidin

Introduction:

This procedure describes the binding of biotinylated ligands such as proteins, peptides or nucleic acids on the **SiMAG-Streptavidin** beads.

The high association constant of Streptavidin / Biotin (10^{15} M^{-1}) guarantee a stable binding of biotinylated compounds with the correct orientation.

SiMAG-Streptavidin are a useful tool for molecular biological application such as isolation of specific DNA sequences and hybrids, cDNA subtraction, or capture of cycle sequencing or PCR reaction products and the isolation of mRNA. Furthermore is applicable for immuno- or protein-based methods or isolation of whole cells, enzyme complexes or organelles.



Equipment and reagents:

- **SiMAG-Streptavidin** (10 mg/ml in PBS, 0.05% sodium azide)
- **Binding Buffer for nucleic acid:**

  10 x SSC (1.5 M NaCl, 0.15 M trisodium citrate, pH 7.0)

- **Binding Buffer for Protein:**

  PBS or TBS (10 mM Tris-HCl, pH 8.0, 150 mM NaCl)

- **Magnetic Separator** (**e.g. MagnetoPURE,** Product Number: MP-10)
- **Binding Capacity:**

Free Biotin:	60 – 250 pmol / mg particles
Biotin-Protein:	40 – 100 pmol / mg particles
Oligonucleotide:	80 – 200 pmol / mg particles

Technical Note:

- Free biotin in the sample will reduce the binding capacity of the **SiMAG-Streptavidin** beads. A disposable column packed with Sephadex can be used to remove of free biotin.
- All biotin reagents should contain a spacer arm to reduce steric hindrance.
- Make sure the **SiMAG-Streptavidin** beads are in suspension during the incubation steps.
- The chemical bond between biotin and streptavidin is incredible strong. It can only be broken under denaturing conditions.
- To elute the bound biotinylated ligand from **SiMAG-Streptavidin**, add appropriate amount of Elution Buffer (10 mM EDTA, pH 8.2, 95% formamide), incubate at 65°C for 2 min, collect the magnetic beads by the Magnetic Separator and transfer the supernatant in a fresh tube.

The following general protocols describe the binding of 1 nmol biotinylated proteins or 2 nmol nucleic acid on 10 mg SiMAG-Streptavidin

Protocols: Binding of biotinylated Protein

1. Resuspended the **SiMAG-Streptavidin** particles by vortexing for 5-10 seconds and transfer 1 ml SiMAG-Streptavidin into a clean 1.5 ml microcentrifuge tube.

2. Wash the particles 3 x with 1 ml PBS before use to remove sodium azid by using the magnetic separator and completely remove and discard the supernatant.

3. After the second wash step resuspend the particles in 0.5 ml PBS.

4. Add 0.5 ml (1 nmol) dissolved biotinylated protein to the particles and mix the suspension on a shaker for 5 - 15 minutes at room temperatur.

5. Wash the bead / protein complex 3 x with 1 ml PBS and collect by using the magnetic separator.

6. Beads are now ready for the desired application or store at 4°C.

Note: For the bound non-biotinylated protein can be eluted by adding of 0.5 ml dH_2O at room temperature for 3-5 minutes. Collect the magnetic beads by the magnetic separator and transfer the supernatant to a fresh tube.

Protocols: Binding of biotinylated nucleic acids

1. Resuspended the **SiMAG-Streptavidin** particles by vortexing for 5-10 seconds and transfer 1 ml **SiMAG-Streptavidin** into a clean 1.5 ml micro centrifuge tube.

2. Wash the particles 3 x with 1 ml SSC before use to remove sodium azide by using the magnetic separator and completely remove and discard the supernatant.

3. After the second wash step resuspend the particles in 0.5 ml SSC.

4. Add 0.5 ml (2 nmol) dissolved biotinylated nucleic acid to the particles and mix the suspension on a shaker for 5 - 15 minutes at room temperatur.

5. Wash the bead / nucleic acid complex 3 x with 1 ml SSC and collect by using the magnetic separator.

6. Beads are now ready for the desired application or store at 4°C.

Note: For the bound of non-biotinylated nucleic acids can be eluted by adding of 0.5 ml dH_2O and heating at 95°C for 3-5 minutes. Collect the magnetic beads by the magnetic separator and transfer the supernatant to a fresh tube.