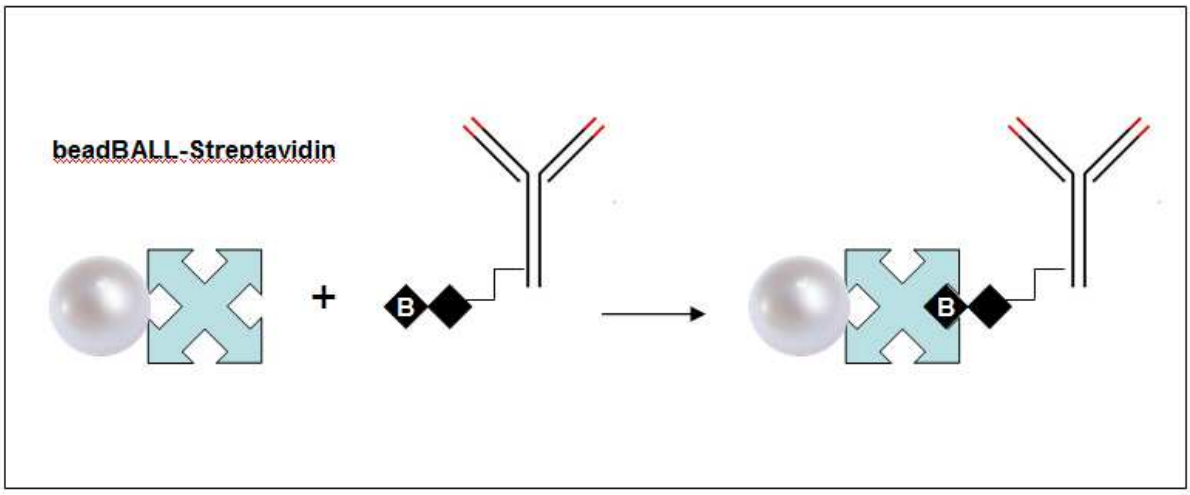
Immobilization of biotinylated ligands with beadBALL-Streptavidin

Introduction:

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

The high association constant of Streptavidin / Biotin (1015 M-1) guarantee a stable binding of biotinylated compounds with the correct orientation.

beadBALL-Streptavidin microspheres are a useful tool for molecular biological applications 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 **beadBALL-Streptavidin** is applicable for immuno- or protein-based methods or enrichment of whole cells, enzyme complexes or organelles.



Equipment and reagents:

- **beadBALL-Streptavidin**
  (10 mg/ml in PBS, 0.05% sodium azide)

- **Protein binding buffer:**
  PBS or TBS (10 mM Tris-HCl, pH 8.0, 150 mM NaCl)

- **Nucleic acid binding buffer:**
  10 x SSC (1.5 M NaCl, 0.15 M trisodium citrate, pH 7.0)

- **Microcentrifuge**

- **Binding capacity:**

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

Technical note:

- Free biotin in the sample will reduce the binding capacity of the **beadBALL-Streptavidin** microspheres. 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 **beadBALL-Streptavidin** microspheres 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 **beadBALL-Streptavidin**, add appropriate amount of elution buffer (10 mM EDTA, pH 8.2, 95% formamide), incubate at 65℃ for 2 minutes, spin down and trans fer the supernatant in a fresh tube.
  Please note: This procedure may irreversible degrade the biotinylated target molecule.

The following protocols describe the binding of 1 nmol biotinylated proteins or 2 nmol nucleic acids on 10 mg beadBALL-Streptavidin.

Protocols: Binding of biotinylated proteins

1. Resuspend the **beadBALL-Streptavidin** microspheres by vortexing for 5 - 10 seconds and transfer 1 ml **beadBALL-Streptavidin** into a clean 2 ml microcentrifuge tube.

2. Spin down and remove the supernatant completely. Completely resuspend the microspheres in 1 ml **Protein binding buffer**, spin down, remove the supernatant and resuspend the microspheres in 0.5 ml **Protein binding buffer**.

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

4. Add 1 ml **Protein binding buffer**, mix, spin down and remove the supernatant. Repeat this step twice. Take care of full resuspension.

5. Microspheres are now ready for the desired application or storage at 4°C.

Note: Non-biotinylated proteins can be eluted as follows. Add of 0.5 ml dH_2O, incubate at room temperature for 3 - 5 minutes, spin down and transfer the supernatant to a fresh tube.

Protocols: Binding of biotinylated nucleic acids

1. Resuspend the **beadBALL-Streptavidin** microspheres by vortexing for 5 - 10 seconds and transfer 1 ml **beadBALL-Streptavidin** into a clean 2 ml microcentrifuge tube.

2. Spin down and remove the supernatant. Completely resuspend the microspheres in 1 ml **Nucleic acid binding buffer**, spin down and remove the supernatant. Repeat this step twice. After the second wash step resuspend the microspheres in 0.5 ml **Nucleic acid binding buffer**.

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

4. Add 1 ml **Nucleic acid binding buffer**, mix, spin down and remove the supernatant. Repeat this step twice. Take care of full resuspension.

5. Microspheres are now ready for the desired application or store at 4°C.

Note: Non-biotinylated nucleic acids can be eluted as follows. Add 0.5 ml dH_2O, incubate at 95°C for 3 - 5 minutes, spin down and t ransfer the supernatant to a fresh tube.