Bangs Laboratories, Inc.
BEADS ABOVE THE REST

Streptavidin-Coated Microspheres Binding Biotinylated DNA

Product Data Sheet 714

DESCRIPTION

The streptavidin-biotin bond is one of the strongest non-covalent bonds ($K=10^{15}/M$) utilized in biological separations. Streptavidin-coated microspheres provide an efficient means of capturing biotinylated PCR products, or binding biotinylated ssDNA or dsDNA for downstream applications. The following protocols were developed using 1µm polymer streptavidin-coated microspheres, and may be adapted for other compositions and diameters.

MATERIAL

Material Required

Streptavidin-coated microspheres
Binding/Wash Buffer: 20mM Tris pH 7.5, 1M NaCl, 1mM EDTA, 0.05% Triton®-X 100 (520µL per binding reaction)
Elution Buffer (as needed): 0.2M NaOH (150µL per reaction)

Instrumentation Required

Microcentrifuge capable of 12,000 x G
Microcentrifuge tubes, 0.5mL-2.0mL
Precision pipets with disposable tips to deliver 1-20µL and 20-200µL
Adjustable vortexer (manual mixing may be used in lieu of vortexing)

PROCEDURE

Researchers are advised to optimize the use of particles in any application.

Binding of Single-stranded or Double-stranded Biotinylated DNA

1. Aliquot 100µL streptavidin-coated microspheres into a microcentrifuge tube.
2. Wash 2 times in 100µL Binding / Wash Buffer by centrifuging the 1µm streptavidin-coated microspheres at 10K rpm for 3 minutes, and decanting the supernatant.
3. Re-suspend the microspheres in 20µL Binding / Wash Buffer and add 5-10µg biotinylated ds or ss oligonucleotide. Keep bead concentration in the range 10-50 mg/mL during the binding step.
4. Incubate 15 minutes at RT on a vortexer (Setting #1), then centrifuge and decant supernatant. (Note: If an adjustable vortexer is unavailable, intermittent manual mixing is advised.)
5. Remove any unbound biotinylated oligonucleotide from the streptavidin-oligo beads by washing 2 times in 100µL Binding / Wash Buffer.
6. Re-suspend in 100µL Binding/Wash Buffer. Oligo-bound microspheres are now ready for downstream applications.

Capture of ds Biotinylated DNA / Elution of ssDNA

Aliquot 100µL streptavidin-coated microspheres into a microcentrifuge tube.

1. Wash 2 times in 100µL Binding / Wash Buffer by centrifuging the 1µm streptavidin-coated microspheres at 10K rpm for 3 minutes, and decanting the supernatant.
2. Re-suspend the microspheres in 20µL Binding / Wash Buffer and add 5-10µg biotinylated double-stranded oligonucleotide (only one strand is biotinylated). Keep bead concentration in the range 10-50 mg/mL during the binding step.
3. Incubate 15 minutes at RT on vortexer (Setting #1), then centrifuge and decant supernatant. (Note: If an adjustable vortexer is unavailable, intermittent manual mixing is advised.)
4. Remove any unbound biotinylated oligonucleotide from the streptavidin-oligo microspheres by washing in 2 times in 100µL Binding / Wash Buffer.
5. To recover the single-stranded DNA, re-suspend the microspheres in 150µL 0.2M NaOH. Agitate the DNA / streptavidin microspheres for 6 minutes at RT on a vortexer or with intermittent manual mixing during the incubation time.
6. Spin and save the eluate containing non-biotinylated single-stranded oligonucleotide DNA.
7. With UV / Vis spectroscopy, read the OD 260 / 280 for determination of the DNA concentration of the eluted sample.

NOTES

1. Quantitation methods for the ss or ds oligonucleotide in the supernatant include absorbance readings via OD 260 / 280 ratios or agarose gel electrophoresis. If the oligonucleotide is labeled with a fluorochrome, flow cytometric analysis may be utilized with an appropriate Quantum™ MESF kit (Catalog Codes 488, 647, 823-828) to quantitate fluorescence intensity of the bound oligonucleotide on the streptavidin microspheres.
2. When binding an oligonucleotide with a fluorochrome, the incubation should be performed in a dark room or with the tube wrapped in foil to avoid photobleaching.

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STORAGE AND STABILITY

Store at 2-8˚C. Freezing of particles may result in irreversible aggregation and loss of binding activity.

Bangs' products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

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