Hydrophilic Streptavidin Magnetic Beads

1-800-632-7799
info@neb.com
www.neb.com

S1421S 0011104 14041

S1421S

5 ml (20 mg) **Lot: 0011104** **Exp: 4/14**

4 mg/ml **Store at 4°C (Do not freeze)**

Description: Hydrophilic Streptavidin Magnetic Beads are 2 µm superparamagnetic particles covalently coupled to a highly pure form of streptavidin. The beads can be used to capture biotin labeled substrates including antigens, antibodies and nucleic acids (1,2). The strength of the biotin-streptavidin interaction, an association constant (Ka) of 10^{15} M^{-1} coupled with the low non-specific binding of streptavidin permits captured substrates to be useful as ligands in subsequent experiments including mRNA isolation and the capture of primary or secondary antibodies (3,4).

Beads are supplied as a 4 mg/ml suspension in phosphate buffer (PBS) (pH 7.4) containing 0.1% BSA and 0.02% NaN_3.

Support Matrix: 2 µM non-porous superparamagnetic microparticle.

Binding Capacity: The beads will bind greater than 800 pmol of free biotin per mg and greater than 400 pmol of single-stranded 20 bp biotinylated oligonucleotide per mg.

Wash/Binding Buffer:
0.5 M NaCl
20 mM Tris-HCl (pH 7.5)
1 mM EDTA

Elution Buffer:
10 mM Tris-HCl (pH 7.5)
1 mM EDTA

Low Salt Buffer:
0.15 M NaCl
20 mM Tris-HCl (pH 7.5)
1 mM EDTA

Protocol

mRNA Isolation using Streptavidin Magnetic Beads: For the isolation of mRNA from 100 µg of total RNA or 5 x 10^6 cells. The yield of poly(A)$^+$ RNA will vary with the type of tissue or cells used.

1. Prepare a 65°C bath.
2. Prewarm Elution Buffer in 70°C bath.
3. Place Low Salt Buffer in ice bath.
4. Dissolve 1.0 A_{260} unit of biotin-$(dT)_{18}$ (NEB #S1325S) in 500 µl of Wash/Binding Buffer. Final concentration 8 pmol/µl.
5. Aliquot 125 µl (500 µg) of Streptavidin Magnetic Beads per 100 µg of total RNA into a clean RNase-free microcentrifuge tube. Add 100 µl of Wash/Binding Buffer and vortex to suspend beads. Apply magnet to side of tube for approximately 30 seconds. Remove and discard supernant.
6. Add 25 µl of biotin-$(dT)_{18}$ solution to magnetic beads and vortex to suspend beads. Incubate at room temperature for 5 minutes with occasional agitation by hand. Apply magnet then remove and discard supernant.
7. Wash beads by adding 100 µl of Wash/Binding Buffer. Vortex to suspend then apply magnet and discard supernant. Repeat wash.
8. Dissolve 100 µg of total RNA in 50 µl of Wash/Binding Buffer and heat at 65°C for 5 minutes Then quickly chill in an ice bath for 3 minutes.
9. Add total RNA sample to previously prepared magnetic beads. Vortex to suspend the particles then incubate at room temperature for 10 minutes with occasional agitation by hand.
10. Apply magnet then remove supernant. Add 100 µl of Wash/Binding Buffer, vortex to suspend beads. Apply magnet then remove and discard supernant. Repeat washing with fresh Wash Buffer.
11. Add 100 µl of cold Low Salt Buffer to beads, vortex to suspend. Apply magnet then remove and discard supernatant.

(See other side)

CERTIFICATE OF ANALYSIS

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8. Dissolve 100 µg of total RNA in 50 µl of Wash/Binding Buffer and heat at 65°C for 5 minutes Then quickly chill in an ice bath for 3 minutes.
9. Add total RNA sample to previously prepared magnetic beads. Vortex to suspend the particles then incubate at room temperature for 10 minutes with occasional agitation by hand.
10. Apply magnet then remove supernant. Add 100 µl of Wash/Binding Buffer, vortex to suspend beads. Apply magnet then remove and discard supernant. Repeat washing with fresh Wash Buffer.
11. Add 100 µl of cold Low Salt Buffer to beads, vortex to suspend. Apply magnet then remove and discard supernatant.

(See other side)

CERTIFICATE OF ANALYSIS

12. Add 25 µl of prewarmed Elution Buffer, vortex to suspend beads then incubate at room temperature for 2 minutes.
13. Apply magnet then transfer supernant to a clean RNase-free microcentrifuge tube.
14. Repeat elution with 25 µl of fresh Elution Buffer. Apply magnet and add supernant to first mRNA elution. At this point quantification of isolated poly(A)$^+$ can be done by spectrophotometric measurement (1 A_{260} = approximately 40 µg) or simply proceed to reverse transcription reaction.

References:

1. Chung, S. et al. (2005) *J. Biol. Chem.*, 280, 4578.
2. Tang, B. et al. (2003) *Genome*, 46, 833-840.
3. Zhang, X. et al. (2006) *J. Clin. Invest.*, 116, 3050-3059.
4. Fernandez-Cabezudo, M.J. et al. (2004) *Internat. Immunol.*, 16, 1215-1223

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