Product Information

Streptavidin-Iron Oxide Particles from *Streptomyces avidinii*

Buffered aqueous suspension

S2415

Product Description

Streptavidin is a 66 kDa homotetrameric protein, isolated from *Streptomyces avidinii*, which, like avidin, has a high affinity for biotin ($K_a \sim 10^{15}$ M^{-1}).[1-4] Streptavidin is slightly anionic (pI ~ 5-6) and non-glycosylated. These properties contribute to its relatively low non-specific binding compared to egg white avidin (a glycoprotein with pI ~ 10.5).[1-3,5] Streptavidin is also more resistant than avidin to dissociation into subunits by guanidinium chloride.[6]

The product is a suspension of iron oxide particles ~1.5 µm in size, which are attached to streptavidin. The particles are suspended in phosphate buffered saline containing 0.1% bovine serum albumin. For various biomolecules, the binding capacity of 1 mg is approximated as follows:

- ~1500 pmoles of free biotin
- 1000 pmoles of 20-mer biotinylated oligonucleotides
- 200 pmoles of a 100-mer biotinylated oligo
- 70 pmoles of a 300 base-pair (300 bp) 5'-biotinylated double-stranded DNA
- 25 pmoles of 1 kilobase (1 kb) 5'-biotinylated double-stranded DNA

Several publications,[7-9] theses,[10] and dissertations[11-12] have cited use of S2415 in their research protocols.

Product Profile

- Concentration: 0.8-1.2 mg/mL (exact value on lot-specific Certificate of Analysis)
- Particles per mL: ~5×10^8
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Storage/Stability

Store this product at 2-8 °C for long-term storage.

Freezing, drying, or centrifuging this product results in extensive aggregation and loss of binding activity. **Do not freeze or dry this product.**

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Shake vigorously before use. Researchers are advised to optimize the use of this product in their applications, because procedures for related products from other manufacturers may not be ideal.

Sample Procedure – Isolation of mRNA

This product can be used in the following protocol to isolate 1-2 µg of messenger RNA (mRNA) from 75-100 µg of total RNA. The total isolation time is less than 30 minutes.

1. Dispense 200 µL of S2415 into a nuclease-free microcentrifuge tube. Using a magnetic separation unit, such as Cat. No. M1167, or a similar rare earth magnetic separator, pull the magnetic particles to the side of the microcentrifuge tube for 30 seconds. Remove and discard the supernatant. Resuspend in 100 µL of binding buffer (20 mM Tris, pH 8.0, 0.5 M NaCl).
2. Incubate 2.5 µL (2.5 µg) of 5'-biotinylated oligo(dT) (or an appropriate amount of biotinylated molecule) with 100 µL of the suspension for 15 minutes at room temperature.
3. Magnetically separate for 30 seconds. Discard the supernatant. Wash the oligo(dT) bound particles with 100 µL of binding buffer twice, leaving the magnetic particles as a wet cake.
4. Bring up the total RNA sample with DEPC-treated water to a total volume of 90 µL.
5. Incubate the RNA sample at 55 °C for 5 minutes to disrupt secondary structures.

6. Add 10 µL of 5 M NaCl to the RNA sample to achieve a final concentration of 0.5 M NaCl.
7. Add the total RNA sample to the washed magnetic particles from Step 3. Mix gently and hybridize at room temperature for three minutes.
8. Magnetically separate and wash the particles with 100 µL of wash buffer (7 mM Tris, pH 8.0, 0.17 M NaCl) twice.
9. Elute the bound mRNA with 25-50 µL of DEPC treated water at 55 °C for two minutes.
10. Magnetically separate. Transfer the supernatant to a nuclease-free microcentrifuge tube.
11. Repeat the elution of mRNA with 25-50 µL of DEPC-treated water at 55 °C for another two minutes, to elute completely the bound mRNA from the particles. Magnetically separate and transfer the supernatant to the tube containing the first elution of mRNA from Step 10.

References

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