Dynabeads™ CaptureSelect™ AAV Magnetic Beads

Catalog Numbers 2853522001, 2853522005, 2853522050, 2853332001, 2853332005, and 2853332050

Pub. No. MAN0028150 Rev. C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product information

Dynabeads™ CaptureSelect™ AAV Magnetic Beads are high-capacity and high-throughput magnetic affinity particles for purification of a broad range of naturally occurring and synthetic adeno-associated virus (AAV) serotypes used for gene therapy applications. The anti-AAVX affinity ligand coupled to the surface of the magnetic beads demonstrates good binding reactivity for various AAV serotypes, including AAV1 to AAV8, AAVrh10, and synthetic serotypes. The anti-AAV9 affinity ligand coupled to the surface of the magnetic beads demonstrates good binding reactivity for the AAV9 serotype. The Dynabeads™ CaptureSelect™ AAV Magnetic Beads enable purification using manual and robotic magnetic separators of AAV serotypes directly from complex source materials in a single step with high purity and yield.

Contents and storage

Table 1 Dynabeads™ CaptureSelect™ AAV Magnetic Beads

Product	Cat. No.	Amount	Storage
Dynabeads™ CaptureSelect™ AAVX Magnetic Beads[1]	2853522001	1 mL	Store at 2–8°C. Do not freeze.
	2853522005	5 mL	
	2853522050	50 mL	
Dynabeads™ CaptureSelect™ AAV9 Magnetic Beads[1]	2853332001	1 mL	Store at 2–8°C. Do not freeze.
	2853332005	5 mL	
	2853332050	50 mL	

[1] Formulated as 4-6% (v/v) slurry in Tris buffered Saline with 0.05% Tween-20 and 0.05% Sodium Azide.

Specifications

Dynabeads™ magnetic beads are uniform, mono-sized superparamagnetic beads, 4.5 µm in diameter, composed of highly cross-linked polystyrene. Each product is formulated as 4-6% (v/v) slurry in Tris buffered Saline with 0.05% Tween-20 and 0.05% Sodium Azide at a concentration of 30 mg beads/mL slurry and 1 mg contains ~1.3 x 10^7 beads. The CaptureSelect™ AAVX affinity ligand or the CaptureSelect™ AAV9 affinity ligand is coupled to the surface, allowing binding of multiple AAV serotypes. Although the AAVX magnetic beads cover a broad range of AAV serotypes, their binding reactivity for AAV9 is minimal. For the purification of the AAV9 capsids, use the AAV9 magnetic beads.

Table 2 Binding specifications, binding capacity and elution conditions

CaptureSelect™ affinity ligand	Binding specificity	Binding capacity/mg of beads	Recommended elution buffer
AAVX	AAV serotypes; AAV1 to AAV8, AAVrh10, synthetic serotypes	~1 x 10^{12} viral particles/mg beads	50 mM citric acid, pH 2.5-3.0
AAV9	AAV9 serotype	>0.5 x 10^{11} viral particles/mg beads	50 mM citric acid, pH 2.5-3.0

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

- Low Protein Binding Collection Tubes, 1.5 mL (Cat. No. 90410)
- AAV serotype cell culture supernatants
- DynaMag™-2 Magnet (Cat. No. 12321D) or equivalent
- Binding/Wash Buffer: Phosphate buffered saline (PBS) consisting of 10 mM phosphate buffer (pH 7.4) with 150 mM NaCl
- Elution buffer: See “Specifications” on page 1
- Neutralization buffer: High ionic strength alkaline buffer (for example, 1 M Tris, pH 8)

Methods

To ensure homogeneity, preferable mix the beads by thoroughly pipetting repeatly or by very gently vortexing (make sure beads do not end up in lid). Do not invert or use rotating platform to homogenize as magnetic beads may end up in the lid. The minimum bead slurry volume that is recommended for AAV purification is 40 µL slurry.

1. Place 40 µL of slurry (1.2 mg beads) of Dynabeads™ CaptureSelect™ AAV Magnetic Beads into a 1.5 mL microcentrifuge tube.
2. Add 460 µL of Binding/Wash buffer to the beads, then gently vortex to mix.
3. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove, then discard the supernatant.
4. Add 0.5 mL of Binding/Wash Buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute.
5. Collect beads with a magnetic stand, then remove and discard the supernatant.
6. Add sample to the washed beads; feed stocks can be applied directly.
   - Add sample that contains up to 1.2 x 10^{12} viral particles to the washed AAVX magnetic beads.
   - Add sample that contains up to 0.6 x 10^{11} viral particles to the washed AAV9 magnetic beads.

   Note: The sample volume can be modified. If the sample volume is <500 µL, dilute it to a final volume of 500 µL with Binding/Wash Buffer.

   Note: (*Optional*) Reserve a small volume of load for subsequent analysis.
7. Add the sample to the tube containing washed magnetic beads, then gently vortex or invert to mix.
8. Incubate the samples at room temperature while mixing at minimal speed for 10–30 minutes. A rotating platform is recommended.
9. Collect the beads with a magnetic stand, then remove the supernatant. The supernatant can be kept for subsequent analysis.
10. Add 500 µL of Binding/Wash Buffer to the tube, mix well, collect the beads with a magnetic stand, then remove the supernatant. Repeat this wash once for a total of two washes.
11. Add 20–50 µL of Elution Buffer to the tube, mix well, then incubate 10 minutes at room temperature with occasional mixing.
12. Collect the beads with a magnetic stand and then remove and save the supernatant that contains the eluted AAV capsids. To neutralize the low pH, add 1 µL of Neutralization Buffer for each 10 µL of eluate.
13. Repeat steps 11 and 12 to ensure complete elution.

    Note: The protocol was set up for small scale AAV capsids purification. The protocol is scalable, but process conditions have to be empirically determined.

Example application with Dynabeads™ CaptureSelect™ AAV Magnetic Beads

For an example of an application run with Dynabeads™ CaptureSelect™ AAVX Magnetic Beads, see Figure 1. A crude AAV5 feedstock sample, with a titer of 1 x 10^{11} viral particles/mL was purified using 40 µL bead slurry/mL feed. The washed beads were incubated for 30 minutes with the sample. After two washing steps with 500 µL PBS, pH 7.4, the beads were eluted with 40 µL 50 mM citric acid, pH 3. The elution step was repeated three times. Samples were collected and analyzed on a silver-stained SDS-PAGE.

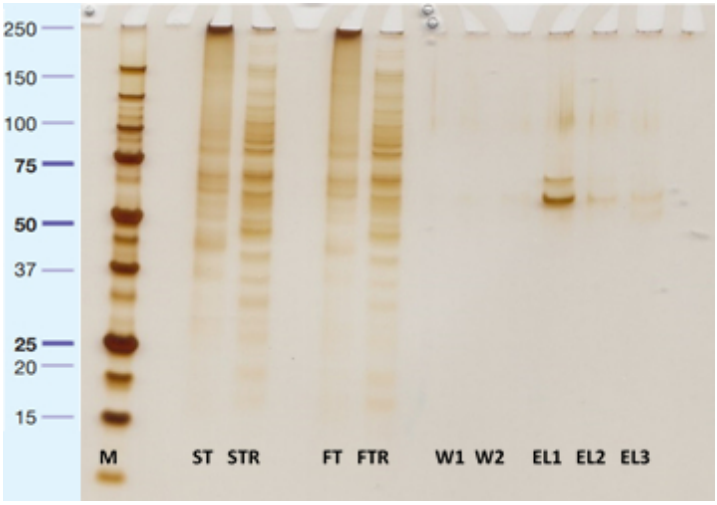

Figure 1 Starting material, flow through, and elution fractions analysis

① M—Molecular weight marker
② ST—AAV start sample (non-reduced)
③ STR—AAV start sample (reduced)
④ FT—Flow through sample (non-reduced)
⑤ FTR—Flow through sample (reduced)
⑥ W1—Wash step 1 (reduced)
⑦ W2—Wash step 2 (reduced)
⑧ EL1—Elution step 1 (reduced)
⑨ EL2—Elution step 2 (reduced)
⑩ EL3—Elution step 3 (reduced)

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

For more information

For more information on CaptureSelect™ products, go to **www.thermofisher.com/captureselect**.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0028150 C.0

Revision	Date	Description
C.0	17 January 2024	Updated Methods introductory paragraph for clarity.
B.0	27 July 2023	• The product information and methods were updated to include Dynabeads™ CaptureSelect™ AAV9 Magnetic Beads. • The document title was updated to Dynabeads™ CaptureSelect™ AAV Magnetic Beads. • The manufacturing address was added.
A.0	6 September 2022	New document for the launch of Dynabeads™ CaptureSelect™ AAVX Magnetic Beads (Cat. Nos. 2853522001, 2853522005, and 2853522050).