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Magnetic ConA Beads for AAV/VLP Purification



Image



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Overview	
Quantity:	250 μL
Application:	Purification (Purif), Separation (Sep)
Product Details	
Purpose:	Immobilization of viruses (e.g. AAV) and virus-like particles (VLP) through Concanavlin A
	binding of capsid glyocproteins.
Characteristics:	 Reversible binding of cells, virus particles, and virus-like particles (VLP) via glycoproteins, glycolipids, or polysaccharides with terminal mannose or glucose. Also suitable for antibody purification. Magnetic ConA Beads (Agarose) for affinity chromatography are based on a ferrimagnetic core surrounded by an agarose matrix covalently bound via polyurethane links. The weak magnetic moment does not interfere with their solubility in the absence of an external magnet field. Upon exposure to a magnetic field however, the beads show a stronger magnetic reaction than the superparamagnetic beads. They are therefore easy to pull out of solution using a magnetic separator.
Components:	10% suspension of Concanavalin A coated agarose particles with a ferrimagnetic core
Bead Ligand:	Concanavalin A
Bead Matrix:	Magnetic Agarose beads
Bead Size:	30 μm
Application Details	
Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	Metal ions (calcium and manganese) mediate the binding to Concanavalin A and stabilize its

conformation.

• The use of buffers with EDTA or other metal chelators must be avoided as it will result in a loss of carbohydrate binding ability.

Protocol:

Suggested buffers:

These buffers create the necessary environment providing bivalent cations for binding (Binding/Wash buffer) and sugar for the release (Elution Buffer) of virus and virus-like particles to the Magnetic ConA Beads for AAV/VLP Purification while maintaining ionic strength and pH. They can be adjusted as needed depending on the experimental setting.

- Binding/Wash Buffer (20 mM HEPES pH 7.5, 10 mM KCl, 1 mM CaCl₂, 1 mM MnCl₂).
- Elution Buffer (100 to 500 mM methyl- α -D-glucopyranoside (methyl- α -D-glucoside) or methyl- α -D-mannopyranoside (methyl- α -D-mannoside), 20 mM HEPES pH 7.5, 10 mM KCl, 1 mM CaCl₂, 1 mM MnCl₂).

Procedure:

This protocol provides a general outline to purify virus or virus-like particles from cell culture supernatant or lysate. Volumes can vary depending on the desired concentration and the expected number of particles in the sample. Please consider the indicated volumes as a starting point and adjust as necessary. Carry out the steps with AAVs or VLPs at 4 °C with cold buffer to minimize potential viral particle degradation or loss of infectivity.

- · Homogenize the Magnetic ConA Beads for AAV/VLP Purification slurry by shaking.
- Transfer 25-50 µL bead slurry to a 1.5 mL microcentrifuge tube for each sample.
- Wash Magnetic ConA Beads for AAV/VLP Purification 3 times with 1 mL Binding Buffer in 1.5 mL microcentrifuge tubes.
- Resuspend beads in a volume of Binding Buffer corresponding to the initial volume of bead slurry.
- Collect the AAV or VLP-containing sample (e.g. cell culture supernatant) in a separate microcentrifuge tube.
- Centrifuge the sample at 1,000 x g for 5 min at 4 °C.
- Transfer the supernatant to a new microcentrifuge tube and centrifuge at 10,000 to 20,000 x g to pellet the virus or virus-like particles.
- Carefully remove the supernatant and resuspend the AAV or VLP-containing pellet in 500 μ L cold binding buffer.
- Add 500 µL of the AAVs or VLPs in binding buffer to the prepared Magnetic ConA Beads for

AAV/VLP Purification in Binding Buffer.

- Mix gently by inverting the tube several times to ensure thorough mixing.
- Incubate the Magnetic ConA Beads for AAV/VLP Purification and AAV or VLP mixture on a rotator or a shaker at 4 °C for 1-2 h.
- Place the tube containing the Magnetic ConA Beads for AAV/VLP Purification and AAV or VLP mixture onto a magnetic separation rack.
- Allow the beads to migrate toward the magnet, and carefully remove and discard the supernatant.
- Remove the tube from the magnetic rack and add 1 mL cold Wash Buffer to the tube containing the Magnetic ConA Beads for AAV/VLP Purification and bound AAVs or VLPs.
- Repeat the washing step at least 3 times for a total of 4 washes to ensure efficient removal
 of contaminants.
- Remove the tube from the magnetic rack and add 100-200 µL Elution Buffer to the Magnetic ConA Beads for AAV/VLP Purification and bound AAVs or VLPs.
- Mix gently to resuspend the Magnetic ConA Beads for AAV/VLP Purification and bound AAVs or VLPs and incubate for 5 min at 4 °C.
- Place the tube back on the magnetic separator to separate the beads from the eluted purified AAVs/VLPs.
- · Carefully transfer the eluted fraction (containing the purified AAVs/VLPs) to a clean tube.
- Optional concentration step: If desired, the purified AAVs/VLPs can be concentrated using an
 appropriate concentration method such as ultracentrifugation or ultrafiltration, according to
 the specific requirements of your downstream applications.
- Store the purified AAVs or VLPs at the recommended storage conditions, usually at -80 °C for long-term storage or -20 °C for shorter-term storage.

Restrictions:

For Research Use only

Handling

Format:	Liquid
Handling Advice:	Do not freeze the Magnetic ConA Beads for AAV/VLP Purification! Vortex bead suspension well before use.
Storage:	4 °C
Expiry Date:	12 months

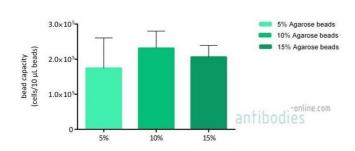


Image 1. Comparison of the number of K562 cells bound to 10 μ L Magnetic ConA Beads (Agarose) slurry at different concentrations. ABIN7446172 is provided as a slurry containing 10% Magnetic ConA Beads (Agarose).