VECTREX® Avidin D

Cat. No. A-2020

VECTREX® Avidin D is one of several systems available from Vector Laboratories for isolating specific sequences of biotinylated nucleic acid or other biotinylated molecules. Other affinity binding systems include Agarose Avidin D (Cat. No. A-2010) and the reversible binding matrices, VECTREX® Avidin DLA (Cat. Nos. MB-2021, MB-2022) or VECTREX® AAL (Aleuria aurantia lectin) (Cat. Nos. MB-1396, MB-1397).

The VECTREX® matrix consists of a highly crosslinked sugar polymer with a very large surface area and low non-specific binding for nucleic acids and other charged macromolecules. This hydrophilic matrix offers an advantage over porous agarose beads because, unlike agarose which may retain small molecules in its pores, the VECTREX® particle will only retain molecules through specific affinity interaction. In addition, the VECTREX® matrix consists of dense particles which sediment readily with centrifugation.

VECTREX® Avidin D is a conjugate of the VECTREX® matrix and a special form of avidin. The extremely high affinity of avidin for biotin makes VECTREX® Avidin D an essentially irreversible binding matrix, useful in affinity purification. For example, after binding of biotinylated nucleic acid to the matrix, nucleic acids with sequences complementary to the bound probes can be selected from a complex pool or sequence-specific binding proteins may be isolated. In addition, many traditional molecular biology techniques can be performed on the immobilized nucleic acids.

Nucleic acids can be biotinylated using the FastTag™ Biotin Labeling Kit (Cat. No. MB-8000), PHOTOPROBE® Biotin (Cat. No. SP-1000), PHOTOPROBE® (Long Arm) Biotin (Cat. No. SP-1020), 5' EndTag™ Labeling Kit (MB-9001), 3' EndTag™ Labeling Kit (MB-9002) or by other established methods. Proteins can be biotinylated with Biotin (Long Arm) NHS (Cat. No. SP-1200), Biotin (Long Arm) NHS, water soluble (Cat. No. SP-1210), Biotin Hydrazide (Cat. No. SP-1100), Biotin (Long Arm) Maleimide (Cat. No. SP-1501) or ProtOn™ Protein Labeling Kit (PLK-1202). Information and protocols for these products are available on our website upon request.

VECTREX® Avidin D is supplied as 2 ml of a 1:1 suspension (vol:vol) in 50 mM sodium bicarbonate, pH 8.2, 150 mM NaCl, 0.08% sodium azide. Store at 4 ˚C.

Binding Protocol:

The following protocol outlines a small-scale batch procedure for binding biotinylated samples to VECTREX® Avidin D. The matrix could also be incorporated into column-based procedures.

1. Centrifuge the appropriate volume of VECTREX® Avidin D slurry* at 12,000 x g for 30 seconds and discard the supernatant. Equilibrate the VECTREX® Avidin D by washing twice with 2 volumes of binding buffer **, centrifuging, and discarding the supernatant following each wash.

* The binding capacity of PHOTOPROBE® Biotin-labeled λ Hind III is approximately 25 ng/µl of 1:1 slurry. The binding capacity for proteins will vary depending on the molecular weight and the number of accessible biotin tags.

** Use TBST (0.1 M Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20) for nucleic acid binding. Use TBS (0.1 M Tris, pH 7.5, 150 mM NaCl) or TBST for protein binding depending on whether detergent is required for protein stability.

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2. Add biotinylated sample (in 1 volume of binding buffer) to the pelleted VECTREX® Avidin D from step 1, gently resuspend, and allow at least 30 minutes for binding at 37 ˚C or 1 hour at room temperature with occasional mixing. (Note: end-labeled nucleic acids or proteins with few biotin tags may require more time for complete binding.)

3. Centrifuge the binding reaction for at least 1 minute at 12,000 x g.

4. Carefully remove the supernatant without disturbing the VECTREX® Avidin D pellet. (Retain the supernatant until binding of the biotinylated sample has been verified in step 7.)

5. Wash the VECTREX® Avidin D matrix with 2 volumes of binding buffer to remove unbound molecules. Pellet the matrix by centrifugation for 1 minute at 12,000 x g and discard the supernatant. Repeat once.

6. Resuspend the VECTREX® Avidin D pellet from step 5 in the desired volume of buffer.

7. Binding of biotinylated nucleic acids may be verified by agarose gel electrophoresis of the supernatant collected in step 4 and staining with ethidium bromide. If the biotinylated sample size is too small or too dilute to be detected with ethidium bromide (≤ 10 ng) but is in the range of 1 pg to 10 ng, the step 4 supernatant can be evaluated by dot blot analysis using Alkaline Phosphatase Streptavidin (Cat. No. SA-5100) and the BCIP/NBT Substrate Kit (Cat. No. SK-5400). The binding of proteins may also be verified by dot blot analysis. (Note: dot blot analysis of proteins must be performed in the absence of Tween 20.)

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