



VECTREX[®] Avidin DLA Binding and Elution Kit Catalog No. MB-2022

The VECTREX[®] Avidin DLA matrix reversibly binds biotinylated molecules. The VECTREX[®] Avidin DLA kit is one of several systems available from Vector Laboratories for isolating biotinylated nucleic acids, proteins, or other biotinylated macromolecules. Recovery of biotinylated probes with their complementary targets or ligands is possible using this system. Other affinity matrices include VECTREX[®] Avidin D (Cat. No. A-2020), Agarose Avidin D (Cat. No. A-2010), and 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.

The Avidin DLA conjugated to the VECTREX[®] matrix is a modified form of Avidin D with a reduced affinity for biotin. This lowered affinity allows VECTREX[®] Avidin DLA to reversibly bind biotin. Biotinylated nucleic acids or other molecules can be bound to VECTREX[®] Avidin DLA and can be subsequently eluted with biotin. Potential applications include probe purification, cDNA and subtraction library construction, hybridization analysis, and *in vitro* mutagenesis. (For reversible binding of molecules to the VECTREX[®] matrix under "physiological" conditions [pH 8.0, 150 mM NaCl] use VECTREX[®] AAL).

Nucleic acids can be biotinylated using the FastTag[®] Biotin Labeling Kits (Cat. No. MB-2000, MB-2001, MB-2002), PHOTOPROBE[®] Biotin (Cat. No. SP-1000), PHOTOPROBE[®] (S-S) Biotin (Cat. No. SP-1010), PHOTOPROBE[®] (Long Arm) Biotin (Cat. No. SP-1020) 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), or Biotin (Long Arm) Maleimide (Cat. No. SP-1501). Information and protocols for these products are available upon request.

VECTREX[®] Avidin DLA is provided as a 1:1 buffered suspension (vol : vol). Store at 4 °C.

Kit Components:

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| 1. 1 ml of settled matrix | 1:1 (vol : vol) in 10 mM HEPES, pH 7.5,
150 mM NaCl, 0.08% (wt/vol) sodium azide |
| 2. 3.0 ml of 10x CENT buffer | 1x = 50 mM trisodium citrate, 1 mM EDTA,
300 mM NaCl, 0.1% (vol/vol) Tween 20, pH 4.0 |
| 3. 2.0 ml of 25 mM biotin solution | 25 mM biotin, 0.75 M Tris, pH 8.0,
0.08% (wt/vol) sodium azide |

Binding and Elution Protocol:

The following protocol outlines a small-scale batch procedure for binding biotinylated samples to VECTREX[®] Avidin DLA and for subsequent elution from the matrix. The matrix could also be incorporated into column-based procedures.

1. Centrifuge the appropriate volume of VECTREX[®] Avidin DLA slurry (*See Note A*) at 12,000 x g for 30 seconds and discard the supernatant. Equilibrate the matrix by washing twice with 2 volumes of 1x CENT (*See Note B*), centrifuging, and discarding the supernatant following each wash.
2. Add biotinylated sample (in 1 volume of 1x CENT) to the pelleted VECTREX[®] Avidin DLA from step 1, gently resuspend, and allow at least 1 hour for binding at 37 °C or 2 hours at room temperature with occasional mixing. *See Note C*.
3. Centrifuge for at least 1 minute at 12,000 x g.
4. Carefully remove the supernatant without disturbing the VECTREX[®] Avidin DLA pellet. (Retain the supernatant until binding of the sample has been verified in step 8.)
5. Wash the VECTREX[®] Avidin DLA matrix with 2 volumes of 1x CENT. Pellet the matrix by centrifugation for 1 minute at 12,000 x g and discard the supernatant. Repeat once.
6. Resuspend the VECTREX[®] Avidin DLA pellet from step 5 in the desired volume of elution buffer. (One to two times the original VECTREX[®] Avidin DLA slurry volume is sufficient.)

Elution buffer (scale volumes up or down as needed):

70 µl H₂O
 10 µl 10x CENT
 20 µl 25 mM biotin solution

Incubate for at least 1 hour at 50 °C or for at least 3 hours at room temperature with occasional mixing. Longer elution times (e.g. overnight) may improve elution efficiency. *See Note C*.

7. Collect the eluted sample (supernatant) after centrifugation at 12,000 x g for 1 minute. Retain the pelleted matrix until elution has been verified in step 8. For maximum recovery of biotinylated sample, perform a second elution and pool the eluates. *See Note D*.
8. Binding and elution of biotinylated nucleic acids should be verified by agarose gel electrophoresis and ethidium bromide staining. If the PHOTOPROBE[®] Biotin- or FastTag[®] Biotin-labeled 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 and step 7 eluate can be evaluated by dot blot analysis using Alkaline Phosphatase Streptavidin (Cat. No. SA-5100) and BCIP/NBT Substrate Kit (Cat. No. SK-5400). The binding and elution of proteins may also be verified by dot blot analysis. (Note: blotting of proteins should be performed in the absence of Tween 20.)

Notes:

- A. *The binding capacity of PHOTOPROBE[®] Biotin-labeled λ Hind III is approximately 25 ng/µl of 1:1 slurry in a 2 hour, 25 °C binding assay. The binding capacity of a 1.8 kb DNA fragment containing a single biotin tag is about 10 ng (8.4 fmol)/µl of 1:1 slurry in a 2 hour, 25 °C binding assay. The binding capacity for proteins will vary depending on the molecular weight and number of accessible biotin tags.*
- B. *If detergents destabilize a biotinylated protein, CENT buffer can be made without Tween 20.*
- C. *Heavily biotinylated molecules (i.e. FastTag[®] Biotin- or PHOTOPROBE[®] Biotin-labeled nucleic acid) will bind quickly but will elute slowly while sparsely biotinylated molecules (i.e. end-labeled nucleic acid) will bind slowly but elute quickly. Some optimization may be necessary if optimal binding and elution efficiencies are required.*
- D. *VECTREX[®] Avidin DLA may be regenerated for reuse by washing extensively with high pH buffer (10 mM Tris, pH 10, 1 mM EDTA, 300 mM NaCl, 0.1% Tween 20) followed by equilibration in CENT buffer. However, since elution of biotinylated samples is not 100%, subsequent samples may become contaminated with previous sample(s).*