



The VECTREX[®] AAL Binding and Elution Kit Catalog No. MB-1397

The VECTREX[®] AAL (*Aleuria aurantia* lectin) matrix reversibly binds fucosylated molecules such as fucosylated DNA, RNA, oligonucleotides, proteins, or other macromolecules. Recovery of fucosylated probes with their complementary targets or ligands is possible using this system. Other affinity systems include VECTREX[®] Avidin D (Cat. No. A-2020), VECTREX[®] Avidin DLA (Cat. Nos. MB-2021 or MB-2022), and Agarose Avidin D (Cat. No. A-2010).

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 *Aleuria aurantia* lectin conjugated to VECTREX[®] particles reversibly binds fucose. Thus fucosylated nucleic acids or other molecules can be bound to VECTREX[®] AAL under a wide variety of conditions and subsequently eluted with fucose under non-denaturing conditions. Potential applications include probe purification, PCR, cDNA and subtraction library construction, hybridization analysis, and *in vitro* mutagenesis.

Nucleic acids can be fucosylated using the FastTag[®] Fucose Labeling Kits (Cat. No. MB-4000 or MB-4001). Information and a protocol for these products are available upon request.

VECTREX[®] AAL is provided as a 1:1 [v:v] buffered suspension. The binding capacity of VECTREX[®] AAL is approximately 25 ng FastTag[®] Fucose- λ /Hind III per μ l of 1:1 slurry, but can vary depending upon the size of the nucleic acid and degree of fucosylation. Nucleic acids labeled with the FastTag[®] Fucose Labeling Kit bind to VECTREX[®] AAL with high specificity in 1x TENT, and can be eluted from the matrix with 1x TENT containing 500 mM fucose.

Kit Components:

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| 1. 1 ml settled matrix | 1:1 (vol:vol) in 10 mM HEPES, pH 7.5, 150 mM NaCl, 0.08% (w/v) sodium azide |
| 2. 3.0 ml of 10x TENT binding buffer | 100 mM Tris, pH 8.0, 10 mM EDTA, 1.5 M NaCl, 1.0% (v/v) Tween 20 |
| 3. 1.0 ml of 1.25 M L-fucose solution | 1.25 M L-fucose in H ₂ O, 0.08% (w/v) sodium azide |

Binding and Elution Protocol:

The following protocol outlines a small-scale batch procedure for binding fucosylated nucleic acid samples to VECTREX[®] AAL and for subsequent elution from the matrix:

1. Centrifuge the appropriate volume of VECTREX[®] AAL slurry (binding capacity is approximately 25 ng fucosylated nucleic acid/ μ l of slurry) at 12,000 x g for 30 seconds and discard the supernatant. Equilibrate the VECTREX[®] AAL by washing twice with two volumes of 1x TENT, centrifuging, and discarding the supernatant following each wash.
2. Add fucosylated nucleic acid (in 1 volume of 1x TENT) to the pelleted VECTREX[®] AAL from step 1, gently resuspend, and allow at least 15 minutes for binding at 37 °C or 30 minutes at room temperature with occasional gentle mixing.
3. Centrifuge the binding reaction for 1 minute at 12,000 x g.
4. Carefully remove the supernatant without disturbing the VECTREX[®] AAL pellet. (Retain the supernatant until complete binding of the nucleic acid sample has been verified in step 8.)
5. Wash the VECTREX[®] AAL matrix with 2 volumes of 1x TENT. Pellet the matrix by centrifugation for 1 minute at 12,000 x g and discard the supernatant. Repeat once.
6. Resuspend the VECTREX[®] AAL pellet from step 5 in the desired volume of elution buffer. One to two times the original VECTREX[®] AAL slurry volume is sufficient.

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

50 μ l H₂O
10 μ l 10x TENT
40 μ l 1.25 M L-fucose

Allow the elution to proceed for at least 15 minutes at 37 °C or for 30 minutes at room temperature with occasional mixing.

7. Collect the eluted nucleic acid (supernatant) by centrifugation at 12, 000 x g for 1 minute. Retain the pelleted matrix until complete elution has been verified in step 8. For maximum recovery of fucosylated nucleic acid, perform a second elution and pool the eluates.
8. Binding and elution of fucosylated nucleic acids should be verified by agarose gel electrophoresis and staining with ethidium bromide. If the fucosylated sample size is too small or too dilute to be detected with ethidium bromide (\leq 10 ng) but is at least 100 pg, the step 4 supernatant and step 7 eluate can be evaluated by dot blot analysis using Alkaline Phosphatase-*Aleuria aurantia* Lectin (Cat. No. MB-4100) and the BCIP/NBT Substrate Kit (Cat. No. SK-5400).