

Detection of Glycoproteins using Lectins in Histochemistry, ELISA, and Western Blot Applications

The following protocols offer guidelines for assay development using lectin-based detection of glycoproteins present in tissue sections, adsorbed onto microtiter plates, or transferred from electrophoretic gels onto nitrocellulose or PVDF membranes.

Histochemistry:

- 1a. Staining procedure for paraffin sections: Deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series and rinse for 5 minutes in tap water. If required, retrieve antigens using an Antigen Unmasking Solution (Citrate-based, H-3300 or Tris-based, H-3301).
- 1b. Staining procedure for frozen sections: Air dry sections. Immediately before staining, fix sections with acetone. Transfer slices to buffer. If endogenous enzyme activities are present, inactivate using appropriate methods.
2. Perform streptavidin/biotin blocking if required following kit instructions using Streptavidin/Biotin Blocking Kit (SP-2002). Avidin in the Avidin/Biotin Blocking Kit (SP-2001) is a glycoprotein containing terminal mannose residues. Use of the Avidin/Biotin Blocking Kit is not recommended especially when using mannose-specific lectins.
3. Block non-specific binding by incubating section with Carbo-Free™ Blocking Solution (SP-5040) for 30 minutes at room temperature. Blot excess blocking solution from the sections.
4. Apply biotinylated lectin at approximately 2-20 µg/ml in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.4) to the sections and incubate for 30 minutes at room temperature. Wash with TPBS (PBS + 0.05% Tween® 20).
5. Prepare VECTASTAIN® Elite® ABC (peroxidase, PK-6100) or VECTASTAIN ABC-AP (alkaline phosphatase, AK-5000) reagents according to the kit instructions. Apply to the sections and incubate for 30 minutes at room temperature. Wash with TPBS.
6. Apply an appropriate precipitating substrate for the enzyme system used in step 4. For peroxidase, ImmPACT® DAB (SK-4105) is recommended; for alkaline phosphatase, ImmPACT Vector® Red (SK-5105) is recommended. Rinse in tap water.
7. Counterstain (optional), clear and mount.

ELISA:

1. Adsorb target protein to microtiter plate by placing 50-200 µl of approximately 3 µg/ml glycoprotein solution into the desired wells. Some wells may be left untreated as negative controls. Incubate at 37 °C for 1 hour. Wash wells three times with TPBS (PBS + 0.05% Tween 20).
2. Block non-specific binding by filling each well to the brim with Carbo-Free Blocking Solution for 30 minutes at room temperature. Wash wells three times with TPBS. The volumes of the reagents in the procedure below are optimized for the development of a 100 cm² membrane. Volumes may be proportionally adjusted for blots of a different size.
3. Apply 50-200 µl of approximately 2-20 µg/ml biotinylated lectin in PBS to the wells and incubate for 30 minutes at room temperature. Wash wells three times with TPBS.
4. Prepare VECTASTAIN Elite ABC or VECTASTAIN ABC-AP reagents according to the kit instructions. Apply to the wells and incubate for 30 minutes at room temperature. Wash wells three times with TPBS.

5. Apply an appropriate non-precipitating substrate for the enzyme system used in step 4. For peroxidase, TMB (SK-4400) is recommended.
6. Quantify the colored reaction product by spectrophotometry.

Western Blot:

1. Perform electrophoresis and transfer proteins to a membrane according to standard procedures.
2. Block non-specific binding by incubating the membrane in Carbo-Free Blocking Solution for 30 minutes at room temperature. Use a sufficient volume to completely cover the membrane.
3. Incubate membrane in PBS containing approximately 2-20 µg/ml biotinylated lectin for 30 minutes at room temperature. Wash with TPBS (PBS +0.05% Tween 20).
4. Prepare VECTASTAIN Elite ABC or VECTASTAIN ABC-AP reagents according to the kit instructions. Incubate the membrane in the reagent for 30 minutes at room temperature. Wash with TPBS.
5. Apply an appropriate substrate for the enzyme system used in step 4. For peroxidase, DuoLuX[®] Chemiluminescent/Fluorescent Substrate for peroxidase (SK-6604) or ImmPACT DAB (SK-4105) are recommended; for alkaline phosphatase, Chemiluminescent/Fluorescent Substrate for alkaline phosphatase (SK-6605) or BCIP/NBT (SK-5400) are recommended.

Negative Controls

Negative controls should be run in parallel in each of the above described methodologies to validate binding results. When applying lectins, one of the most appropriate negative controls is to preabsorb the lectin with a concentration of a defined sugar, with which, the lectin has a known high affinity. Vector Labs offers a series of sugars that are intended for such a purpose.

The lectin is diluted to a suitable working concentration in a solution containing approximately 200 mM to 500 mM of the sugar. This mixture is left to bind at room temperature for 30 to 60 min. Following this absorption incubation, the mixture is substituted into the procedure in place of the unabsorbed lectin and incubated under the same conditions. The subsequent detection procedure is followed as for the test method. In most cases the vast majority of lectin binding to the tissue section (membrane blot, etc.) will be eliminated. Some trace binding to the section (blot etc) may still be present under these conditions and probably indicates presence of secondary or tertiary sugar preferences. These negative control results should be compared with the test results to determine specificity of binding.