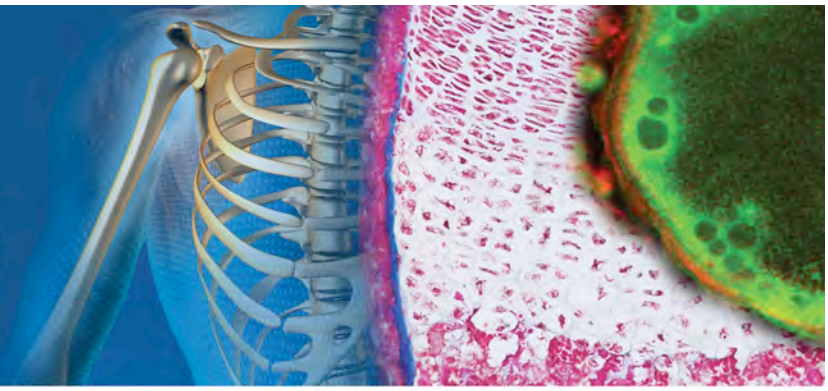

Cadherin11

Invitrogen cat #71-7600
Immunohistochemistry Protocol For Formalin Fixed Paraffin Embedded Tissue
Cut sections at 3 microns and bake overnight at 60°C

Day 1

1. Bake slides at **60°C for minimum of 30 minutes** prior to staining.
2. Deparaffinize tissues in xylene (3 changes, 5 minutes each), and rehydrate through graded alcohols (2 changes in 100%, and 95%, 1 change 70% for 5 minutes each).
3. Wash twice in deionized water for 5 minutes each.
4. Antigen Retrieval is performed in a water bath with a **1X Dako Antigen Retrieval Solution** (*Dako S1699*) **at 70°C for 1 hour.** After the incubation, let the slides cool down to room temperature for about 20 minutes.
5. Outline each section with a **PAP pen.**
6. Quench the endogenous peroxidase activity with **DAKO Dual Endogenous Enzyme Blocking Reagent (***Dako S2003***) for 30 min.**
7. Rinse the slides in 3 changes of deionized water and once in 1X PBST.
8. Block non-specific binding sites **with 1:20 Vector Normal Goat Serum (***Vector S1000***) – incubate for 30 min.**
9. Drain off the normal serum. Do not rinse or wash it off the slides.
10. Block endogenous biotin **with the BEAT Blocking Solutions (***Invitrogen cat# 50-300)* Add 2 drops of blocking solution **1A** to each section and **incubate for 30 min.**
11. Rinse the sections in deionized water.
12. Then rinse the sections **twice** in 1X PBST **for 3 minutes each.**
13. Add 2 drops of blocking solution **1B** to each section and **incubate for 10 min.**
14. Then rinse the sections **twice** in 1X PBST **for 3 minutes each.**
15. Prepare the primary antibody in **1:50 Normal Goat Serum**, this decreases non-specific staining. Negative control slides need to be incubated only with 1:50 normal goat serum**.**
16. Incubate the slides **overnight at 4°C** with a **1:200** dilution of **Cadherin11 primary antibody** *(Invitrogen cat# 71-7600)* **for 2-3 month old adult sections.**

Day 2

1. Let slides warm up to room temperature for 30 minutes, then rinse **5 times with 1X PBST for 5 minutes each.**
2. Incubate with a **1:200** dilution of **Vector Biotinylated Goat anti-rabbit** secondary antibody **(***Vector BA-1000)* for **30 min.**
3. Wash 5 times with 1X PBST for 5 minutes each.
4. Incubate with a **1:250** dilution **Streptavidin-HRP reagent** *(Thermo Scientific cat#21130)* for **30 min.**
5. Wash 4 times with 1X PBST, then twice in deionized water for 5 minutes each.
6. Detect color reaction with **Vector Immpact DAB** *(Vector SK-4105)* for a few minutes (check under microscope)
7. Stop the reaction with deionized water.
8. Counterstain the sections with **Hematoxylin (***Zymed Cat # 93-3943***)** for 5 minutes.
9. Rinse in tap water.
10. Place slides in 1X PBS for 1-3 minutes.
11. Rinse with deionized water.
12. Dehydrate quickly through 95% ethanol (3 changes) and 2 changes of 100% ethanol.
13. Clear in 3 changes of xylene and mount with Cytoseal.

Revised on 7/15/2011 by Ashish Thomas, M.S.