

# UAB Neuroscience Molecular Detection Core

## REAGENTS FOR IMMUNOHISTOCHEMISTRY

### 10X Phosphate Buffered Saline (PBS), pH7.2: (Store at room temperature)

80.0 grams NaCl

2.0 grams KCl

14.2 grams Na<sub>2</sub>HPO<sub>4</sub> (anhydrous)

2.0 grams KH<sub>2</sub>PO<sub>4</sub>,

0.1 gram NaN<sub>3</sub> (optional as a preservative)

Make to 1 Liter with distilled H<sub>2</sub>O.

Adjust pH to 7.2–7.3 Use at 1X

### 10X Tris Buffer (Tris-HCl)

121.0 g Tris

500 ml distilled H<sub>2</sub>O

Adjust pH to 7.6 with HCl

Make to 1 Liter with distilled H<sub>2</sub>O. Use at 1X .

### 'Universal' PBS-Blocking Buffer (Store at 4°C):

1.0 gram bovine serum albumin

0.2 gram non-fat powdered skim milk (e.g. Carnation)

0.3 ml Triton X-100

100 ml 1X PBS

Mix vigorously until clear. Filter sterilize. Store 4° C.

### Blocking Reagents for Goat antibodies (Store at 4°C):

Block and secondary solution:

5% Horse or donkey serum in 0.3% Triton-X100 in PBS; filtered.

Primary antibodies raised in goat are incubated in filtered 0.3% Triton-X100 in PBS.

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## Nuclear Counterstains

- bisBenzimide: Hoechst 33258 ([Sigma](#)–Aldrich, code: B2883)
- Hematoxylin: Harris Modified Hematoxylin with Acetic Acid (Mercury–Free) [Fisher Scientific](#), SH26–500D
- Others are available suitable for various applications from many sources e.g. [Invitrogen](#)

## Antigen Retrieval Solutions (Store at 4°C):

- 10mM Citrate Buffer: pH 6.0:  
Component A: 0.1 M Citric Acid, monohydrate (2.1 gram/100 ml).  
Component B: 0.1 M Sodium Citrate (14.75gram/500 ml).  
Citrate Working solution consists of:  
9 ml of 0.1M Citric Acid (Component A)  
41 ml of 0.1M Sodium Citrate (Component B) and  
450 ml of distilled H<sub>2</sub>O.
- Target Retrieval Solution, High pH, [Dako](#), Carpinteria, CA, 93013 code: S3308

## DETECTION TECHNOLOGIES AND REAGENTS

- TSA Reagent kits ([Perkin–Elmer Life Sciences](#), Store at 4°C):  
Standard TSA Systems, code: NEL700001KT etc.  
Plus TSA Systems code: NEL 741001KT, etc.
- Q Dots™ nanocrystal conjugates ([Invitrogen](#), Carlsbad, CA)  
Available conjugated to Streptavidin and secondary antibodies
- Vectastain™ Elite ABC kit, [Vector Laboratories](#), Burlingame, CA, 94010,  
code: PK–6100
- DAB–Metal Enhanced DAB kit, [Pierce](#), Rockford, IL 61105 code:34065
- StableDAB Peroxidase Substrate, [KPL](#), Gaithersburg, MD 20879, code: 54–11–00

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## Additional Reagents:

- Primary Antibodies: Monoclonal or polyclonal (various sources).
- Secondary Antibodies: Affinipure™ conjugated Whole IgG, [Jackson ImmunoResearch](#), West Grove, PA.
- AutoFluorescence Eliminator Reagent: [Millipore Corp](#), code:2160
- Clearing Agent: CitriSolve [Fisher Scientific](#), code: 22-143975
- Humidity chambers can be made with strips of moistened paper towels and plastic slide folders. [VWR Scientific](#), code: 48443-850
- Mounting media
  - Cytoseal™ 60 toluene based permanent mounting media [Richard Allen Scientific](#) code:8310-4
  - PBS-Glycerol: equal parts 1X PBS and Glycerin. [Fisher Scientific](#), code: BP G33 (90% Glycerin in PBS can be used for Quantum Dot preservation)
- Hydrophobic slide pens: Super PAP Pen ([RPI Corporation](#), Mount Prospect, IL, code: 195505 and 195506), ImmEdge ([Vector Laboratories](#), Burlingame, CA code: H4000). 'Liquid Blocker' pen ([Sigma](#)-Aldrich code: Z377821 and Z672548)

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