REAGENTS FOR IMMUNOHISTOCHEMISTRY

10X Phosphate Buffered Saline (PBS), pH 7.2: (Store at room temperature)
80.0 grams NaCl
2.0 grams KCl
14.2 grams Na₂HPO₄ (anhydrous)
2.0 grams KH₂PO₄,
0.1 gram NaN₃ (optional as a preservative)
Make to 1 Liter with distilled H₂O.
Adjust pH to 7.2–7.3 Use at 1X

10X Tris Buffer (Tris–HCl)
121.0 g Tris
500 ml distilled H₂O
Adjust pH to 7.6 with HCl
Make to 1 Liter with distilled H₂O. 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.
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 (14.75 gram/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 H₂O.
- 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 Strepavidin 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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