



## CLINICAL IMMUNOHISTOCHEMISTRY - MATERIALS & REAGENTS Preparation and Storage

### Materials:

Amber bottles - Wheaton 1000 mL (Fisher, 02-911-147)  
Carboy, Nalgene 5 1/4 gallon (Fisher, 02-961-55C)  
Centrifuge tubes, 15 mL capacity (VWR, 21008-918)  
Centrifuge tubes, 50 mL capacity conical tubes (VWR, 21008-951)  
Coplin jars with lids (Fisher, 08-813D and 08-813E)  
Eppendorf pipettors (Various: 1 uL - 1,000 uL working range)  
Erlenmeyer flask, 500mL (2-3) (Fisher, 10-040H)  
Erlenmeyer flasks, 1000 mL (2-3) (Fisher, 10-040K)  
Gauze (Stat-lab, 7623)  
Graduated cylinders 100 mL(2-3) (Fisher, 08-572D)  
Graduated cylinders, 1000 mL (2-3) (Fisher, 08-572G)  
InfraRed Hot Plate (Fisher, IR6100)  
Kim Wipes (Fisher, 06-666A)  
Lab Wipes (Stat-Lab, SL-5790)  
Latex Gloves (VWR, 37001-646) or Nitrile Gloves (Fisher, 11-388-30)  
Microcentrifuge tubes, 1.5 mL (Fisher, 05-07-5)  
Pipet Aid Drummond (Fisher, 13-681-19)  
Pipet Aid replacement filters (Fisher, 13-681-18E)  
Pipet tips for each pipettor (0.1 uL - 1,000 uL working range)  
Pipets, 10 mL (Fisher, 13-678-25E)  
Pipets, 5 mL (Fisher, 13-678-25D)  
Plexy-glass humidity chambers (1-2) (UTSAHSC, Custom)  
Polypropylene forceps (VWR, 30049-122)  
Pressure Cooker, T-Fal (5qt)  
Sequenza Cover plates (Thermo-Shandon, 72110013)  
Sequenza Staining Racks (Thermo-Shandon, 73310017)  
Super-Slip Cover Glass (24x40) (Fisher, 1254587)  
Tissue-Tek slide baskets (vertical) (VWR, 25609-868)  
Tissue-Tek staining dishes (green and clear) (VWR, 25608-904)  
Transfer pipettes (2-3 mL) (Fisher, 13-711-9A)  
Varistainer XL 24-4 (Thermo-Shandon, 74200011)

### Reagents and Chemicals:

#### **Ammonia water**

Nanopure water	700 mL
Ammonium hydroxide (Fisher)	0.75 mL (750 uL)

Make fresh once a week

#### **Acid alcohol**

70% Ethanol	1000 mL
Hydrochloric acid, conc. (Fisher)	2.5 mL

Make fresh once a week

#### **Agar, 4% purified (Sigma, A-7049)**

Using a 250 mL Erlenmeyer flask, dissolve the following:

Agar, purified (Sigma, A-7049)	4 g.
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Sodium Azide, (Fisher, S227-100)	0.05 g
NPW (see <i>Nanopure</i> below)	100 mL

Heat in the microwave for 1 minute, stir the solution, replace the solution in the microwave and heat for 30 more seconds, stir again and reheat for a final 30 seconds (at this point agar solution becomes viscous and boils).

Quickly aliquot the solution into Dolphin tubes with the Drummond Automatic Pipetter and a 10 mL glass pipette. Tubes are to be filled to ~80% capacity and then chilled in the refrigerator. Once the agar hardens, the tubes may be transferred to the -70°C freezer for storage.

One hour before use, necessary aliquots for processing the cell lines may be removed from storage and heated on a dry bath at 95°C, loosen the cap to avoid pressure build up. Replace the tube cap and re-suspend the agar by vortexing. The molten agar is now ready for use.

#### Antibody diluent\*

Using a 250 mL Erlenmeyer flask, dissolve the following:

Bovine albumin (Sigma, A-2153)	1 g
Sodium azide ((Fisher, S227-100))	0.1 g
1X TBS-20	100 mL

Adjust pH to 7.6 with 10 N NaOH or 6 N HCL.

\*Store at 4-6°C (solution is stable for 2 months).

#### Avidin-Biotin Blocking Kit (Avidin A and Avidin B solutions) Ready to Use (Vector Laboratories, SP2001)

#### Boric acid, 2 mM

Using a 1000 mL Erlenmeyer flask, dissolve the following:

a. Boric acid, FW 61.83 (EM science, BX0865-1)	0.124 g.
b. NPW	1000 mL

#### Citrate Buffer, 0.01 M, pH 6.0 (C6) and Citrate Buffer 0.01 M, pH 3.0 (C3)

Using a 100 mL Erlenmeyer flask, dissolve the following:

##### a. 0.1M Citric acid solution\*

Citric acid, MW 210.14	21.01 g
NPW	1000 mL

Using a 100 mL Erlenmeyer flask, dissolve the following:

##### b. 0.1M Sodium citrate Solution\*

Sodium citrate, MW 294.12	29.4 g
NPW	1000 mL

\*Store both solutions at 4-6°C (solution is stable for 1 month).

Using a graduated cylinder mix in the following proportions:

19 mL of a + 81 mL of b + 900 mL of NPW = **0.01M Citrate Buffer (C6) adjust pH to 6.0 if needed.\*\***



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93 mL of a + 7 mL of b + 900 mL of NPW= **0.01M Citrate Buffer (C3) adjust pH to 3.0, if needed.\*\***

\*\*Store both solutions at 4-6°C (solution is stable for 1 month).

#### **Diaminobenzidine solution, 3, 3 (DAB+)\* Working (CARCINOGEN = use precautions)**

Using the DAB plus kit, (Dako, K 3468), add 1 drop of the chromogen for every 1 mL for substrate of working DAB to be used, and mix thoroughly.

\*Store at 4-6°C (solution is stable for 1 week).

#### **EDTA, 0.01M (= 10mM), pH 8.0\***

Using a 1000 mL Erlenmeyer flask, dissolve the following:

EDTA, disodium salt (Sigma, ED2SS)	3.72 g.
NPW	1000 mL

Adjust pH to 8.0 with NaOH. Adjust pH very carefully for the pH may change abruptly as it approaches 8.0.

\*Store at Room temperature (solution is stable for 6 months).

#### **Ethanol, 100%**

Purchased from the Baylor College of Medicine Stock Room, Integrated Services

#### **Ethanol, 95%**

Purchased from the Baylor College of Medicine Stock Room, Integrated Services

#### **Ethanol, 70%\***

In a Nalgene 5 1/4 gallon carboy, mix the following:

100% Ethanol	7000 mL
NPW	3000 mL

\*Store at room temperature

#### **Ficin**

Ficin, (Sigma, F-4125)

Shake well and dilute to 0.71 units/mL in 1X TBS with Tween-20. Digest sections for 20 minutes at room temperature using sequenza coverplates. Remove coverplates and rinse in NPWx10. Transfer sections to TBS-20

#### **Harris Acidified Hematoxylin (Thermo-Shandon, 6765003)**

#### **Hybridization solution**

In a 15 mL capacity centrifuge tube, mix the following:

Formamide (50%)	5 mL
50% Dextran sulphate (10%)	2 mL
20X SSC (5X)	2.5 mL
1M Sodium phosphate pH 6.5 (25mM)	0.25 mL
50X Denhardt's (1X)	0.2 mL
Nanopure Water	0.038 mL
Sonicated salmon sperm DNA (0.25 mg/mL)	0.258 mL



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### Hydrochloric acid (6M or 6N)\*

Using a 100 mL graduated cylinder mix the following:

Hydrochloric acid (Fisher, A144s-500)	24.79 mL
NPW	25.21 mL

\*Store at room temperature (solution is stable for 2 months).

### Hydrochloric acid (1 M or 1N)\*

Using a 1000 mL Erlenmeyer flask, mix the following:

Hydrochloric acid (Fisher, A144s-500)	50 mL
NPW	555 mL

\*Store at room temperature (solution is stable for 2 months).

### Hydrogen Peroxide, 3% Working\*

To a graduated cylinder, add:

TBS-20	180 mL
30% Hydrogen peroxide stock (Sigma, H1009)	20 mL

\*Prepared fresh daily.

### Methyl green solution, 0.05% (MG 0.05%)\*

Using a 1000 mL Erlenmeyer flask, dissolve the following:

Methyl green, CV-free (Sigma, M-6776)	0.150 g
NPW	300 mL

\*Store at Room temperature (solution is stable for 1 week).

### Mouse IgG, Biotin labeled, made in Rabbit (Dako, E0354) (MIgG-Biotin (Rabbit) Dako)

See appropriate assay for dilution.

### Nanopure water (NPW) \*

Deionized water is also acceptable in the absence of NPW, as the laboratory's standard is declared as Type II.

\*Store at room temperature (solution is stable for no more than 2 weeks).

### Osmium tetroxide, 0.2% \* (TOXIC = use precautions)

In a Wheaton amber bottle (1000 mL capacity) break a sealed 10 mL ampule of 4% osmium tetroxide stock solution (Sigma, O-0631) and add 190 mL of NPW.

\*Store in the dark at Room temperature (solution is stable for 2 weeks).

### Permaslip Coverslipping Media (Stat-Lab)

### Phosphate buffered saline, 0.01 M (PBS)\*

Using a 1000 mL Erlenmeyer flask, dissolve the following:

0.01M PBS pH 7.4 (Sigma, P-3813)	1 Pack
NPW	1000 mL

\*Store at 4-6°C (solution is stable for 2 weeks).



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### **Pronase\***

Using a 250 mL Erlenmeyer flask, dissolve the following:

Pronase** (Calbiochem 53702)	0.01
1X TBS w/o Tween-20	100 mL

Mix, digest sections using a coplin jar at room temperature for 5 minutes. Rinse in several changes of TBS.

\*Discard after each use.

\*\*Store at 4-6°C (solution is stable for 2 weeks).

### **Sodium Hydroxide, (10M or 10N)\***

Using a 1000 mL Erlenmeyer flask, dissolve the following:

Sodium Hydroxide (Fisher, S318-3)	40 g.
NPW	100 mL

\*Store at room temperature (solution is stable for 1 year).

### **Sodium Hydroxide , (1M or 1N )\***

Using a 1000 mL Erlenmeyer flask, dissolve the following:

10 N Sodium Hydroxide (Prepared above)	100 mL.
NPW	900 mL

\*Store at room temperature (solution is stable for 1 year).

### **Streptavidin, Horseradish Peroxidase Labeled (Dako, P0397), (LSA-HRP)**

See appropriate assay for dilution.

### **Target Retrieval Solution, Working 1X (TRS)\***

Using a 500 mL graduated cylinder mix the following:

a.	10X Target Retrieval Solution (Dako, S1699)	25 mL
b.	NPW	225 mL

\*Discard immediately after each use.

### **Tris Buffered Saline at pH 7.6 (TBS)**

#### 10X Tris buffered Saline stock solution (10X TBS)\*

Using a 1000 mL Erlenmeyer flask, dissolve the following:

Tris Base (Sigma, T-1503)	14 g.
Tris HCl (Sigma, T-3253)	60 g.
Sodium chloride (Fisher, BP 358-1)	87.5 g.
Nanopure water( NPW)	1000 mL

#### 1X Tris buffered Saline working solution (TBS)\*\*

Dilute 100 mL of 10X TBS in 900 mL of NPW.



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Note: Measure daily and adjust pH of solution to 7.6 ( $\pm$  0.02) if required and document the data in the Quality Control Notebook (QCN). Use 1M HCl to decrease or 1M NaOH to increase pH.

\*Store at 4-6°C (solution is stable for 6 months).

\*\*Store at 4-6°C (solution is stable for 2 weeks).

#### Tris Buffered Saline + Tween 20 at pH 7.6 (TBS-20)

10X Tris buffered Saline stock solution (10X TBS-20)\*

Using a 1000 mL Erlenmeyer flask, dissolve the following:

Tris Base (Sigma, T-1503)	14 g.
Tris HCl (Sigma, T-3253)	60 g.
Tween 20 (Fisher, BP337-500)	5 mL
Sodium chloride (Fisher, BP 358-1)	87.5 g.
Nanopure water (NPW)**	1000 mL

1X Tris buffered Saline working solution (TBS-20)\*\*\*

Dilute 100 mL of 10X TBS-20 in 900 mL of NPW.

Note: Measure daily and adjust pH of solution to 7.6 ( $\pm$  0.02) if required and document the data in the Quality Control Notebook (QCN). Use 1M HCl to decrease or 1M NaOH to increase pH.

\*Store at 4-6°C (solution is stable for 6 months).

\*\*\*Store at 4-6°C (solution is stable for 2 weeks).

#### Tris-EDTA pH 8.0, (TE8)

1 M Tris-HCl buffer

Using a 100 mL Erlenmeyer flask, dissolve the following:

a) Tris Base	12.1 g
NPW	100 mL

Using a 100 mL Erlenmeyer flask, dissolve the following:

b) Tris-HCl	15.76 g
NPW	100 mL

Mix a & b in the following proportions:

61.24 mL of **a** + 38.76 mL of **b** = 100X Tris HCl buffer pH 8

Add 3.74 g of EDTA for a **100X 0.1M Tris-EDTA\***

Dilute immediately prior to use at 1:100 for a **1X Tris-EDTA solution, adjust pH 8.0 if needed and discard after each use.**

\*Store at room temperature (solution is stable for 1 year).

#### Tris-HCl Buffer at pH 9.0, 0.1 M (Tris-HCL 9 AR Buffer)

Solution A: 0.2M Tris Base\*

Using a 1000 mL Erlenmeyer flask, dissolve the following:

Tris Base	24.2 g
NPW	1000 mL



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\*Store at room temperature (solution is stable for 2 months).

### Solution B: 0.2M HCl\*

Using a 1000 mL Erlenmeyer flask, dissolve the following:

Hydrochloric acid (Fisher, A144s-500)	16.52 mL
NPW	983.48 mL

\*Store at room temperature (solution is stable for 2 months).

### Working 0.1M Tris-HCl Buffer at pH 9.0\*

Solution A	100 mL
Solution B	10 mL
NPW	110 mL
Tween-20	220 uL

\*Prepare fresh daily, adjust pH of solution to 9.0 ( $\pm 0.02$ ) if required, and document the data in the Quality Control Notebook (QCN). Use 6N HCl to decrease or 1M NaOH to increase pH.

### **Trypsin\***

Using a 1mL cylinder test tube, dissolve the following:

- a. Trypsin Tablet 1 mg  
(Type II: Crude from porcine, Sigma T-7168)
- b. NPW 1 mL

\*Mix, digest sections at 37°C for 20 minutes. Rinse in several changes of TBS. Discard after each use.

### **Urea\***

Using a 1000 mL Erlenmeyer flask, dissolve the following:

- a. Urea (Sigma, U5128) 100 g.
- b. NPW 1000 mL

\*Store at Room temperature (solution is stable for 1 year).

### **Xylenes, ACS, (Fisher, X5S-4) or (Fisher, X5-4)**

Fisher X5S-4 is preferred to minimize the use of breakable containers.