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.
**Preparation and Storage**

**Sodium Azide, (Fisher, S227-100)** 0.05 g  
**NPW (see Nanopure 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:

- Boric acid, FW 61.83 (EM science, BX0865-1) 0.124 g.  
- 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:

- **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:

- **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) (MlgG-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 Coverslapping 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).*
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 (± 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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base (Sigma, T-1503)</td>
<td>14 g.</td>
</tr>
<tr>
<td>Tris HCl (Sigma, T-3253)</td>
<td>60 g.</td>
</tr>
<tr>
<td>Tween 20 (Fisher, BP337-500)</td>
<td>5 mL</td>
</tr>
<tr>
<td>Sodium chloride (Fisher, BP 358-1)</td>
<td>87.5 g.</td>
</tr>
<tr>
<td>Nanopure water (NPW)**</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

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 (± 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
   NPW

Using a 100 mL Erlenmeyer flask, dissolve the following:

b) Tris-HCl
   NPW

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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base</td>
<td>24.2 g</td>
</tr>
<tr>
<td>NPW</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>
**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**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A</td>
<td>100 mL</td>
</tr>
<tr>
<td>Solution B</td>
<td>10 mL</td>
</tr>
<tr>
<td>NPW</td>
<td>110 mL</td>
</tr>
<tr>
<td>Tween-20</td>
<td>220 uL</td>
</tr>
</tbody>
</table>

*Prepare fresh daily, adjust pH of solution to 9.0 (± 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:

- Trypsin Tablet (Type II: Crude from porcine, Sigma T-7168) 1 mg
- 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:

- Urea (Sigma, U5128) 100 g.
- 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.