

	SOP-BCR-5.5	Immunohistochemistry Staining (IHC)	Authors: S. Clouthier  Ahmed "Ali" Quaraishi Approved: M. Wicha 	Rev: 1	Issued: 7/17/09 Revised: 6/12/2013
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1.0 Purpose




The purpose of SOP 5.5 is to provide information on Immunohistochemistry Staining (IHC) using Invitrogen Histostain Plus IHC Kit.

2.0 Scope

SOP 5.5 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Procedure

- 3.1 First, load slides into staining rack (grey rack from green holders) for each of the "dips."
- 3.2 Dip slides in Xylene. Every step of soaking or washing should be done in 10 dips. The dips help remove wax on slides. Do this step for 5 minutes and repeat it three times.
- 3.3 Next, dip the slides 10 times in 100% EtOH, and then let it sit in 100% EtOH for five minutes. Repeat this step two times. This step removes the Xylene.
- 3.4 Dip Slides 10 times in 95% EtOH and let them sit for five minutes. This step should be completed once.
- 3.5 Dip slides 10 times in 80% EtOH and let them sit for five minutes. This step should be completed once.
- 3.6 Put the holder with slides in flowing distilled water for five minutes. Do not let the flow of water directly hit the slides.
- 3.7 Pour out distilled water, and add Citrate Buffer to slide holder. Make sure the slides are submerged and completely cover the top of slides with the Citrate Buffer.
- 3.8 Microwave the slides for 10 minutes, then six minutes on power level seven. The slides should be microwaved for a total of 16 minutes in the pH 6.0 Citrate Buffer. Add more citrate buffer after the first 10 minutes, then microwave another six minutes.
- 3.9 Next, cool down the slides for 10 minutes. Pour the Citrate Buffer back into the original container to reuse. Place the container full of slides in flowing tap water. Do not let the tap water touch the slides! Only allow the water to flow into the container holding slides.
- 3.10 For five minutes, allow distilled water to flow into the container of slides. Do not let the distilled water directly hit the slides!
- 3.11 Dip the slides into PBS 1x. Let this stand for two minutes.
- 3.12 Make 25mL 30% H₂O₂ (Sigma bottle located in fridge #1) + 225 mL Methanol to fill container (4 L Fisher container in flammable cabinet). The H₂O₂ is reusable, therefore please pour back into glass container and put in Fridge #1 when done. Slides should soak in this mixture for ten minutes.
- 3.13 Dip the slides into PBS for two minutes. Repeat this step two times. (This step is optional).
- 3.14 Wipe off the excess PBS with a Kimwipe.
- 3.15 Wet the bottom of the staining tray (Styrofoam with pipettes across it to hold slides)- so stains don't try up.
- 3.16 Take an Immedge Pen or DakoPen and draw a circle around the tissue. Make sure to give enough space around the tissue. After drawing, wait until it dries. Make sure the tissue sample doesn't dry!
- 3.17 Take the **Blue Color Blocking Solution** from the Histo Kit in Fridge #1, and put this on top of the tissue, one slide after another. Let this sit for 10 minutes.

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- 3.18 Tap off the solution. Do not wash off!
- 3.19 Take a Kimwipe and clean around the tissue.
- 3.20 Next, using 1' Ab, mix the antibody with 1X PBS at dilution stated in the technical data sheet. For example, for ER, 1:50 dilution is 1 µL of antibody to 50 µL of PBS. Apply the primary antibody to the slides one by one adding just enough to cover the tissue. Do not wipe off after incubation, just tap off excess. This can be done for one hour at room temperature, or, overnight at 4°C.
- 3.21 Dip slides in PBS1X and let sit for two minutes. Repeat this step two times.
- 3.22 Then, using 2' Ab, the yellow color from the Histo Kit, cover the tissue with this. If Rat 2'Ab (Abcam) is used, incubate for 30 minutes (1:500). Otherwise, let this sit for 10 minutes.
- 3.23 Dip slides in PBS1X and let sit for two minutes. Repeat this step two times.
- 3.24 Using HRP, the red color in the Histo Kit, put this solution on the tissue one slide after another.
- 3.25 Dip slides in PBS1X and let sit for two minutes. Repeat this step two times.
- 3.26 Use DAB (for longer term fixing) or AEC (will not stay fixed/set for long) for 1-5 minutes. DAB, NovaRED, BCIP/NBT are not dissolved by EtOH, and is used for longer term fixing. (Permunt can be used).
 - If using AEC- Does not stay fix/set for long period of time, mount with Glycerol.
- 3.27 Dip the slides in Distilled Water for three minutes. Repeat this step three times. Remember to dip slides 10 times before letting them soak in the distilled water.
- 3.28 Dip the slides into Hematoxylin for 30 seconds to one minute.
- 3.29 Dip the slides into Tap water for three minutes.
 - Please note: when using AEC, mount now with glycerol- don't do any more steps from here for AEC/Glycerol mounting!
- 3.30 Dip slides 10 times in 100% EtOH. Repeat this step twice.
 - Please note: Only do this step for DAB, NovaRED, BCIP/NBT staining!
- 3.31 Dip the slides in Xylene for five minutes. Repeat this step three times.
- 3.32 Permunt, Histomount, Surgipath- using Permunt, put drops over stain, then put the cover slide over and squish so all bubbles are out from under the cover slide. Lay slide flat to dry.

4.0 Applicable References

5.0 Change Description

Revision	Date	Reference	Description of Change