

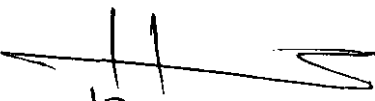
**IN SITU HYBRIDIZATION PROTOCOL**  
**AUTORADIOGRAPHY OF SLIDES**

1. Place the dipping chamber and a vial of emulsion in a 41-42 C water bath for 30 minutes to warm. Next pour the emulsion slowly down the side of the chamber so that no bubbles form.
2. Dip the slides and place them on end in a slide rack.
3. Allow the slides to **dry** for at least 3 hours, preferably overnight. A non-sparking hairdryer can be used to reduce this time to 1-2 hours.
4. Store the dried slides in light-tight plastic boxes. Seal the boxes with black electrical tape and store these light-proof containers at 4C for exposure to occur. Moisture will cause fading of the latent image, and with very weak signals, the fading of the image can be nearly equal to its production. However, if the slides are kept very dry, even weak signals can be detected after a long exposure. The autoradiographic exposure time may range from a few hours to many days. Therefore, it is necessary to have several replicate slides so that test slides can be developed at different intervals in order to determine the correct exposure time. The average time for exposure is 10-12 days.

**DEVELOPING THE SLIDES:**

5. Although the exact temperature is not critical, it is best to develop the autoradiographs at 16-17C. The lower the temperature, the smaller the grain size. However, it is more important that the slides and all of the developer and fixer solutions are at the same temperature since temperature changes may produce wrinkles in the emulsion. The schedule for developing autoradiographs is:
  - A) KODAK D19 developer: 3.5 minutes  
with constant agitation
  - B) Water: 30 seconds
  - C) KODAK rapid fixer: 5 minutes (with some agitation)
  - D) Running tap water: 20 minutes (this step can be carried out in daylight)

NOTE: Tap water can be very cold in the winter. Try to adjust temperature of water to between 15-20C.
6. While the slides are in the developer, agitate them gently by rocking the dish back and forth for cycles of 6 second movement and 6 second rest. The movement ensures that the developer is mixed and does not become depleted near areas of high grain density on the slides. Slides should also be agitated gently in the other solutions from time to time. However, the amount of agitation is not as critical as during the incubation in the developer.
7. After the final rinse, clear the slides of all extra emulsion. The area behind the tissue should be clean. Rinse well in water in order to remove all emulsion particles.
8. Counterstain slides in haematoxylin as follows:
  - A) Haematoxyllin Gil II: 10 seconds (with agitation)
  - B) Rinse well in tap water until water becomes clear
  - C) Saturated Lithium Carbonate: 20 seconds (with agitation)
  - D) Rinse well in tap water.



9. Dehydrate the slides in increasing concentration of alcohol as listed below:

A) Alcohol 90% : 30 seconds - 2 minutes

B) Alcohol 100% : 30 seconds - 2 minutes

C) Alcohol 100% : 30 seconds - 2 minutes

D) Xylene : 30 seconds - 2 minutes

10. Apply Entellan mounting medium to slides and place a cover slip on top.

11. Allow the slides to air dry and then label the slides with regards to tissue time, experiment #, etc.

## AUTORADIOGRAPHY- DEVELOPING.

	<u>For S-35</u>	<u>For P-32</u>
Temperature:	16° - 17° C	18° -19° C
Developing sol.	3.5 min.	5 min.
ddH <sub>2</sub> O	30 sec.	30 sec.
Rapid Fixer	5 min.	5 min.
Running Tap H <sub>2</sub> O	20 min.	20 min.

- Clean slides with a razorblade to remove all extra emulsion, especially the area behind the tissue.
- Rinse slides well, to remove any loose particles which otherwise might end up in the Haematoxylin dish.
- Stain and dehydrate slides and coverslip.
- Let dry before putting under the microscope, so as not to clog up the lenses.

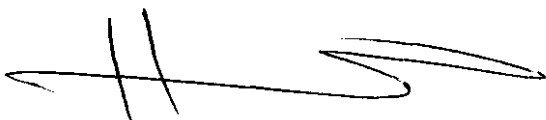
### DEVELOPING STOCK SOLUTION

- Kodak D-19 powder - 1 package makes 3.8 Liter - Is too much.
- Weigh off 297.50 gram for 1.9 Liter dd H<sub>2</sub>O.Or,148.75 gr./950 ml.
- Put in 2 Lt. flask, cover with aluminum foil (must be protected from light )
- Stir until dissolved ( ~ 1 Hour ) Solution is lightbrown.
- To remove color, filter through Whatman paper #1, solution should now be clear.
- Aliquot in 250 ml brown bottles, up to the neck.
- **When solution turns brown after storage, please discard.** It is not good anymore for developing ISH slides

### KODAK RAPID FIXER SOLUTION

dd H <sub>2</sub> O	950 ml
sol. A	473 ml
Add sol. B	52 ml
dd H <sub>2</sub> O	425 ml

1. Mix 950 ml water and solution A.
2. Add solution B.
3. Agitate well to prevent precipitation.
4. Add 425 ml water.



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