North2South Chemiluminescent Detection

Fisher catalog # 17097
https://www.thermofisher.com/order/catalog/product/17097
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Note: *Volumes may change depending on size of membrane & containers used*.
Rinse forceps between each step.

1. The day before (ie after starting hybridization) bring Hybridization Stringency Wash Buffer (2X) to room temperature- warm to 37^0C if there is still a precipitate. Then make dilution for needed volume. For 1 blot: 40 mls of 2X buffer plus 40 mls of sterile H_2O. This wash buffer can be left in a water bath overnight at the hybridization temperature. Note: *Stringency of wash buffer can be adjusted (0.1X to 2X)*.

2. The next day, put Blocking Buffer, 4X Blocking Wash Buffer, & sterile water in a 37^0C water bath. Put Substrate Equilibration Buffer, Peroxide Solution, & Luminol/Enhancer Solution at room temperature.

3. Pour off hybridization solution. Wash membrane with "1X" Hyb washing buffer.
   a. 25 mls, 15-20 minutes, hyb temp, shaking or rotating
   b. 25 mls, 15-20 minutes, hyb temp, shaking or rotating
   c. 25 mls, 15-20 minutes, hyb temp, shaking or rotating

4. Put membrane in a clean box (slightly larger than the membrane).
   a. Add 16 mls of Blocking Buffer.
   b. Shake gently, room temperature, 15 minutes

5. During step 4, mix 16 mls of Blocking Buffer + 55 ul of the strep/HRP conjugate.
   a. After the 15 minute incubation in step 4, decant blocking buffer.
   b. Add Blocking Buffer + conjugate.
   c. Shake gently, room temperature, 15 minutes.

6. During step 5, make 1X Blocking Wash Buffer. For 1 membrane, mix 25 mls of prewarmed 4X Wash buffer and 75 mls of pre-warmed H_2O. Keep 1X buffer at 37^0C.
   a. After the 15 minute incubation in step 5, transfer membrane to a clean box.
   b. Rinse briefly with 20 mls of 1X block wash.
   c. Wash membrane 20 mls, 5 minutes, gentle shaking, room temp
   d. Wash membrane 20 mls, 5 minutes, gentle shaking, room temp
   e. Wash membrane 20 mls, 5 minutes, gentle shaking, room temp
   f. Wash membrane 20 mls, 5 minutes, gentle shaking, room temp

7. Transfer membrane to a clean box.
   a. Wash membrane 5 minutes in Substrate Equilibration Buffer, gentle shaking, room temperature

8. Mix 6 mls luminol/enhancer and 6 mls peroxide solutions. Protect from light during this and following steps.
   a. Pour mixture into clean container
   b. Using clean forceps, pick up membrane, blot one corner on filter paper for 3 seconds, place membrane DNA/RNA side down onto the luminol/proxide "puddle".
   c. Incubate 5 minutes, no shaking, room temperature.
   d. Pick up membrane with forceps, blot one corner on filter paper for 3 seconds, wrap in saran wrap.

9. Expose membrane to CCD camera. Adjust exposure length as needed.