Whole-mount in situ Hybridization

Supplement for simultaneous detection of differently labeled riboprobes

8/15/94 Kenji Shimamura

1. Prepare digoxigenin-labeled and fluorescein-labeled riboprobes. (digoxigenin- and fluorescein-labeled UTP are available from Boheringer Mannhein)

2. Purify the transcripts by precipitation with lithium chloride and ethanol, or by spin-column with Sephadex G-50 beads.

3. Hybridize with the mixture of probes (0.5-1 μg/ml each).

4. Wash probes.

5. Incubate with pre absorbed anti-fluorescein alkaline phosphatase conjugated antibodies (Boheringer-Mannheim) or anti-digoxigenin antibodies.

6. Wash antibodies.

7. Incubate with *BCIP/NBT substrate, or Vector black alkaline phosphatase substrate (Vector Laboratories).

8. Wash with CMFET.

9. Inactivate enzyme completely by heating (70°C, 30 min), by dehydration through methanol, or by incubation with 0.1M glycin-HCl pH2.2 for 10 min.

10. Wash with TBST.

11. Incubate with the other antibodies.

12. Develop color reaction with the other set of substrate.

*BCIP/NBT gives dark blue signal, whereas Vector black substrate gives dark brown staining.

INT/BCIP that produces brilliant red precipitates is available from Boheringer.