

## Protocol P *In Situ* Hybridisation Protocol for PRINSKit 1490-T

To be used with the detection kit appropriate to the label.  
No detection necessary with FITC or Cy3 label.

| Solutions:       | Stop Buffer                   |
|------------------|-------------------------------|
| Stop Buffer (1X) | 50mM NaCl<br>50mMEDTA, pH 8.0 |

Reaction mix contains dNTPs, primer and glycerol.

### Procedure

1. Make chromosome preparations according to standard protocols  
Slides can be made up to 24 hr in advance and stored at 4°C
2. Combine 50µl of reaction mix, 1nmole of Cy3 or FITC-dUTP or 0.04mmoles biotin-dUTP and 2 units of Taq polymerase per slide  
**Note:** *These are not supplied with the probe*
3. Add to slide. Seal with Cow Gum and dry for 5 min in an oven at 37°C
4. Place slide on a preheated hot block at 94°C for 5 min
5. Transfer to an oven at 58°C for 30 min
6. Wash in stop buffer for 5 min at 58°C
7. Wash in stop buffer for 5 min at room temp
8. Dehydrate through an ethanol series and air dry
9. Dilute 1µl of Counterstain 1 (DAPI) with 9µl distilled water. Add 5µl diluted counterstain to 200µl of mountant. Mix well
10. Mount slides with 20µl of mountant/counterstain. Overlay with a coverslip and seal with nail varnish
11. Slides should be stored in the dark at 4°C
12. View slides using epifluorescence filters as appropriate

**Note:** *These products are not for use in humans and are for research purposes only*

Troubleshooting tips are  
included in Section 3