Nick Translation (FISH)

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Reagents

Bovine serum albumin (BSA)
dATP, dTTP, dGTP, dCTP
Boehringer Mannheim, Cat. 105 1440, 105 1458, 105 1466, 105 1482
DNase I from bovine pancreas
Boehringer Mannheim, Cat.104 159, 100 mg
dUTP (conjugated to hapten or fluorochrome of choice)
EDTA, 0.5 M
Glycerol
Lambda HindIII DNA marker
Magnesium chloride ($MgCl_2$), 0.5 M
β–Mercaptoethanol, 99%
Polymerase (Kornberg)
Boehringer Mannheim, Cat. 104 485
NaCl, 1 M
Tris-HCl, 1 M, pH 8.0
Water, sterile

Preparation

dNTP

100 mM dATP, dCTP, and dGTP	5 µl of each	f.c. [0.5 mM]
100 mM dTTP	1 µl	f.c. [0.1 mM]
Sterile water	984 µl	
Total	1000 µl	

(equals 0.5 mM each of dATP, dCTP, and dGTP, and 0.05 mM dTTP)
*Aliquot and store at -20°C

DNase I stock solution, 1mg/ml

DNase I	10 mg	
NaCl, 1M	1.5 ml	f.c. [0.15 M]
Glycerol	5 ml	f.c. [50%]
Sterile water	bring up to 10 ml	

*Aliquot and store at -20°C

10X NT-Buffer

Tris-HCL, 1 M, pH 8.0	500 µl	f.c. [0.5 M]
$MgCl_2$, 0.5 M	100 µl	f.c. [50 mM]
BSA, 10 mg/ml	50 µl	f.c. [0.5 mg.ml]
Sterile water	350 µl	
Total	1000 µl	

*Aliquot and store at -20°C

0.1M ß-Mercaptoethanol

99% solution (14.4 M)		34.7 µl
Sterile water	bring up to	5 ml

*Aliquot and store at -20°C

Procedure

1. For each DNA sample, add to an eppendorph tube:
   2 µg DNA
   10 µl 10X NT-Buffer
   10 µl dNTP
   10 µl 0.1 M ß-Mercaptoethanol
   4 µl BIO-16-dUTP or 4 µl DIG-11-dUTP (1 mM)
   X µl sterile water
   (The total volume including reagents added in step 3 should be 100 µl)

2. Vortex, centrifuge, and place tubes on ice.

3. Add 2 µl Polymerase (Kornberg) first, and then 3-8 µl Dnase (1 mg/ml) diluted 1:1000

4. Flick tube to mix.

5. Incubate at 15°C for 1.5-2 hr.

6. Prepare gel electrophoresis.

7. Run 5 µl of each sample with loading buffer and the Lambda HindIII DNA marker; ideally the length of the DNA should be 500-900 bp for chromosome paint probes or 300-600 bp for gene specific probes after nick translation.

8. If DNA is too large, add more DNase and incubate at 15°C for 10-30 min.

9. Stop the nick translation with 1 µl of 0.5 M EDTA and incubate at 65°C for 10 min.

10. Store DNA at -20°C or precipitate the same day for hybridization.