

Nick Translation (FISH)

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Biotin-16-dUTP

Boehringer Mannheim, Cat. 1093070

Bovine serum albumin (BSA)

Dig-11-dUTP

Boehringer Mannheim, Cat. 1093088

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

EDTA, 0.5 M

Glycerol

Lambda HindIII DNA marker

Magnesium chloride (MgCl₂), 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

100 mM dTTP 1 µl

Sterile water 984 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 μ l f.c. [0.5 M] |
| MgCl ₂ , 0.5 M | 100 μ l f.c. [50 mM] |
| BSA, 10 mg/ml | 50 μ l f.c. [0.5 mg.ml] |
| Sterile water | 350 μ l |

Aliquot and store at -20°C

0.1M β -Mercaptoethanol

| | |
|-----------------------|------------------|
| 99% solution (14.4 M) | 34.7 μ l |
| 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 μ g DNA
 - 10 μ l 10X NT-Buffer
 - 10 μ l dNTP
 - 10 μ l 0.1 M β -Mercaptoethanol
 - 4 μ l BIO-16-dUTP or 4 μ l DIG-11-dUTP (1 mM)
 - X μ l sterile water(The total volume including reagents added in step 3 should be 100 μ l)
2. Vortex, centrifuge, and place tubes on ice.
3. Add 2 μ l Polymerase (Kornberg) first, and then 3-8 μ l Dnase (1 mg/ml) 1:1000
4. Vortex and centrifuge.
5. Incubate at 15°C for 2 hr (1.5-2 hr).
6. Prepare gel electrophoresis.
7. Run about 5 μ l of each sample and the Lambda HindIII DNA marker;
ideally the length of the DNA should be 500-900 bp 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 μ l of 0.5 M EDTA and incubate at 65°C for 10 min.
10. Store DNA at -20°C or precipitate the same day.