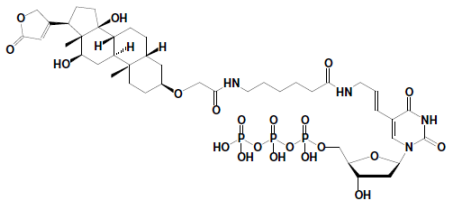
FT-DY1160

Digoxigenin-11-dUTP

Product Description

.

Name :	**Digoxigenin-11-dUTP** Digoxigenin-X-5-Aminoallyl-2'-deoxy-uridine-5'-triphosphate, Triethylammonium salt
Catalog Number :	FP-DY1160, 25 µl (1mM) FP-DY1161, 125 µl (1mM)
Structure :	$C_{43}H_{65}N_4O_{21}P_3$ (free acid)
Molecular Weight :	MW= 1066.91 (free acid)
Purity:	> 95%, clear aqueous solution, pH 7.5
Absorption :	λ_{max} = 290 nm
EC (M^{-1} cm^{-1}) **:**	8780



Storage: -20°C (12 months after date of delivery). Protect from light and moisture
Short term exposure (up to 1 week cumulative) to ambient temperature possible.

Directions for use

Applications

- Nick translation
- PCR

Nick Translation Labeling

Reagents

1. 10x nick translation buffer (0.5M Tris.Cl pH 7.5, 0.1M MgSO4, 1mM dithiotreitol, 500 µg/ml BSA) (Tris, 500g, #1840; NaCl, 1 kg, MgSO4, 1 kg, DTT, 1g, BSA, 5%)
2. 0.5 mM dNTPs : 2µl of each 100 mM dATP, dCTP and dGTP plus 1194µl sterile distilled water (store at –20°C)
3. 1 mM digoxigenin-11-dUTP
4. 1mM dTTP : 1 µl of 100 mM dTTP plus 99 µl sterile distilled water (store at -20°C)
5. DNA polymerase I (500 U, 10U/µl)
6. DNase I stock solution : 1 mg/ml (50 mg DNase I in 50 ml 0.15 M NaCl/50% glycerol; aliquot 1 ml; store at -20°C)
   DNase I working solution: 1 µg/ml
7. 0.5M EDTA (dissolve 186.1 g EDTA and 20 g NaOH in 900 ml distilled water; adjust pH to 7.4 using 6N HCl; bring total volume to 1 liter ; sterilize by autoclave; aliquot 1 ml; store at -20°C)
8. 3M sodium acetate pH 5.6 (dissolve 24.61 g sodium acetate in 90ml distilled water; adjust pH to 5.6 using acetic acid 1N; bring total volume to 100 ml; sterilize by autoclave; store at 4°C)
9. denatured en sonicated fish sperm DNA
10. absolute ethanol, stored at –20°C
11. 80% ethanol, stored at –20°C

Method

1. Take a 1.5 ml microcentrifuge tube (on ice) and add in following order :
   • 2.5 µl 10x nick translation buffer
   • x µl sterile distilled water (to make final volume of 25µl = 18.7µl - x µl DNase I - y µl DNA)

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- 1.9 µl 0.5 mM dNTP mix
- 0.7 µl 1 mM digoxigenin-11-dUTP
- 0.7 µl 1 mM dTTP
- x µl DNase I working solution (or other volume determined by titration)
  - YACs: 2 µl voor 40'
  - BACs/PACs: 1.4 µl voor 40'
  - P1: 1.4 µl voor 40'
  - Cosmids: 1.2 µl voor 40'
  - Alu-PCR: 1.0 µl voor 40'
  - Plasmids: 1.0 µl voor 40'
- 0.5 µl DNA polymerase I (5 units)
- y µl DNA (usually 1 µg)

2. Mix well by flicking the tube. Centrifuge briefly.
3. Incubate at 14°C for the desired time (usually 40 minutes)
4. Add 1/10th volume 0.5M EDTA to inactivate the enzymes
5. Add 1/10th volume 3M sodium acetate pH 5.6, 1ml ice-cold 100% ethanol and 2 µl fish sperm DNA to precipitate the DNA. Mix thoroughly by inversion.
6. Incubate at –70°C for 30 minutes (or –20°C overnight)
7. Centrifuge at 4°C, 14.000 rpm for ½ hour. A pellet should be clearly visible, discard the supernatant
8. Add 1ml of ice-cold 80% ethanol, without disturbing the pellet, centrifuge immediately for 10 minutes: 14.000 rpm, 4°C. Discard the supernatant immediately, leaving the pellet as dry as possible. The pellet will now be transparent and difficult to see (the pellet tends to loosen and the labeled probe may be accidentally discarded)
9. Air dry pellet, but do not over-dry
10. Add 10 µl TE buffer and stand on ice 10 minutes. Flick mix to resuspend probe. Store at –20°C until required.

Other protocol may be found in the literature.

References

- **McCreery**. *et al.*, Digoxigenin labeling, *Mol Biotechnol.* 7(2):121 (1997) Abstract

- **Jackson MP**. *et al*. Detection of Shiga toxin-producing Shigella dysenteriae type 1 and Escherichia coli by using polymerase chain reaction with incorporation of digoxigenin-11-dUTP, *J Clin Microbiol*. 29(9):1910-4 (1991) Article

Technical and scientific information

Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.

- Biotin-16-UTP, FP-AM551A

Ordering information

Catalog size quantities and prices may be found at www.interchim.com/
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06