invitrogen
by life technologies™

Human Cot-1 DNA®

Cat. No.	Size:
15279-011	500 µg
15279-101	1 mg
Conc: 1 mg/ml	Store at -20°C

Description

Human Cot-1 DNA® is obtained from human placental DNA by extracting, shearing, denaturing, and reannealing DNA under conditions that enrich for repetitive DNA sequences such as the *Alu* I and *Kpn* I families (1,2). Human Cot-1 DNA® can be used to suppress cross-hybridization (3,4) to human repetitive DNA when human DNA probes (i.e., cosmids, YACs, and chromosome-painting probes) are hybridized *in situ*. It can also be used to suppress cross-hybridization to human repetitive DNA during filter-hybridization experiments. Human Cot-1 DNA® itself can be labeled to provide an effective hybridization probe to check for the presence of human DNA (i.e., checking cosmids or YACs obtained from human hybrid libraries).

Note on concentration: The concentration of the Human Cot-1 DNA® provided with catalog number 15279-101 is verified by fluorometry.

Labeling Human Cot-1 DNA®

Probes can be labeled with ^{32}P by random primer or nick translation procedures using the Random Primers DNA Labeling System (Cat. No. 18187-013) or Nick Translation System (Cat. No. 18160-010). Biotinylated Cot-1 DNA® can be prepared by nick translation with the BioNick™ Labeling System (Cat. No. 18247-015) or by the BioPrime® DNA Labeling System (Cat. No. 18094-011). Improved results are obtained when the Cot-1 DNA® is first ligated to itself to provide an optimum template.

Storage Buffer

10 mM Tris-HCl (pH 7.4), 1 mM EDTA

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For Research Use Only. Not for diagnostic procedures.

Preparation of probes suppressed with Cot-1 DNA®

The optimum amount of Cot-1 DNA® required to obtain effective suppression of repetitive DNA sequence hybridization will depend on the specific application, and the type and amount of probe DNA. Titration of probes in increasing amounts of Cot-1 DNA® or extending the time for prehybridization may be required to establish optimal suppression for certain probes.

In situ hybridization:

Human Cot-1 DNA® can be used in the range of 0.1 µg/µl to 1.0 µg/µl. Initial experiments using 0.1 µg/µl are recommended. Additional descriptions of *in situ* hybridization methods using various probes can be found in the literature (3-6).

1. Combine labeled probe DNA and Cot-1 DNA®. Add 1/10 volume of 3 M sodium acetate (pH 5.5) and 2 volumes of ethanol. Place tube at -70°C for 30 minutes or at -20°C overnight. Centrifuge the tube, remove ethanol, wash with 70% ethanol, and dry pellet. Alternatively, the Human Cot-1 DNA® can be concentrated by ethanol precipitation separately to 10 mg/ml and can be added directly to the hybridization solution.
2. Dissolve DNAs in 50% formamide, 2X SSC, and 10% dextran sulfate, and vortex extensively.
3. Denature the probe/Cot-1 DNA® mixture by heating at 70°C for 5 minutes.
4. Incubate probe/Cot-1 DNA® mixture at 37°C for 20 minutes.
5. Denature metaphase chromosomes by incubating slides in 70% formamide in 2X SSC at 70°C for 2 minutes.
6. Add probe/Cot-1 DNA® mixture, apply coverslip, seal with rubber cement and hybridize at the appropriate temperature (usually 37°C, but this may have to be optimized for the particular probe used).
7. Wash and process the slides using procedures appropriate for the detection method (fluorescent, enzymatic, or radioactive detection).

Filter hybridization:

1. After the probe (plasmid, lambda, cosmid, YAC) DNA has been labeled, centrifuge the reaction products through a 1-ml bed of Sephadex® G-50 for 2 minutes at 1500 × *g* to remove unincorporated nucleotides. Adjust final probe volume to 100 µl using TE buffer [10 mM Tris-HCl (pH 7.4) 1 mM EDTA].
2. Concentrate Cot-1 DNA® (supplied at 1 mg/ml) by ethanol precipitation to 10 mg/ml. Remove desired amount of Cot-1 DNA® and add 1/10 volume of 3 M sodium acetate (pH 5.5) and 2 volumes of ethanol. Mix, chill 10 minutes in a dry ice/ethanol bath, and centrifuge. Dry the pellet and resuspend in TE buffer to 1/10 of original volume (for a final concentration of Human Cot-1 DNA® of 10 mg/ml).
3. To the labeled probe (25 to 500 ng DNA in 100 µl) add 5 µl of concentrated unlabeled Cot-1 DNA® (50 µg), 50 µl of 20X SSC, 25 µl water, and 20 µl of 1% (w/v) SDS, and mix well.
4. Denature probe/Cot-1 DNA® mixture by placing tube(s) into boiling water for 5 minutes.
5. Transfer tube(s) to a 65°C water bath and incubate for at least 20 minutes.
6. Add probe/Cot-1 DNA® mixture to filter(s).

Quality Control

Catalog number 15279-011: Purity, DNA size, and concentration are verified by spectrophotometry and agarose gel electrophoresis.

Catalog number 15279-101: Purity, DNA size, and concentration are verified by fluorometry and agarose gel electrophoresis.

References

1. Weiner, A.M., et al. (1986) *Ann. Rev. Biochem. 55*, 631.
2. Britten, R.J., et al. (1986) *Methods Enzymol. 29*, 363.
3. Landegent, J.E., et al. (1986) *Hum. Genet. 77*, 366.
4. Lengauer, C., et al. (1990) *Hum. Genet. 86*, 1.
5. Lichter P, et al. (1988) *Hum. Genet. 80*, 224.
6. Lichter P, et al. (1990) *Science 247*, 64-9.

Caution: The human-source raw material used in the production of this procedure tested negative for hepatitis B virus, hepatitis C virus (HCV), human immunodeficiency virus type-1 (HIV-1) and type-2 (HIV-2), human T-cell lymphotropic virus (HTLV-1 and HTLV-2) and *Treponema pallidum*. Handle as if potentially infectious.

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