invitrogen
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Mouse COT-1 DNA®

Cat No. 18440-016 **Size: 500 µg**
Conc.: 1 mg/ml **Store at -20°C.**

Description:
Mouse COT-1 DNA is obtained from Hsd:ICR(CD-1®) mice by extracting, shearing, denaturing, and reannealing DNA under conditions that enrich for repetitive DNA sequences such as the B1, B2, and L1 families (1,2). Mouse COT-1 DNA can be used to suppress cross-hybridization (3,4) to mouse repetitive DNA when mouse DNA probes (*i.e*, cosmids, YACs, and chromosome painting probes) are hybridized *in situ*. It can also be used to suppress cross-hybridization to mouse repetitive DNA during filter hybridization experiments. Mouse COT-1 DNA itself can be labeled to provide an effective hybridization probe to check for the presence of mouse DNA (*i.e*, checking cosmids or YACs obtained from mouse hybrid libraries).

Storage Buffer:
10 mM Tris-HCl (pH 7.4)
1 mM EDTA

Quality Control:
Purity, concentration and DNA size were verified spectrophotometrically and by agarose gel electrophoresis.

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Protocols:

Labeling Mouse COT-1 DNA:

Probes can be labeled with ^{32}P by random primer or nick translation procedures using the Random Primers DNA Labeling System or Nick Translation System. Biotinylated COT-1 DNA can be prepared by nick translation with the BIONICK™ Labeling System or by the BIOPRIME® DNA Labeling System.

Improved results are obtained when the COT-1 DNA is first ligated to itself to provide an optimum template.

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.

A. Preparation of probes suppressed with COT-1 DNA for *in situ* hybridizations:

Mouse 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 Mouse 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 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).

B. Preparation of probes suppressed with COT-1 DNA for 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 (10 mM Tris-HCl, pH 7.4, 1 mM EDTA) 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).

References:

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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.
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