User Guide

Version 1.0 (April 2010)

Product:	**FISH-grade *cot***
Description:	Human C_0t-1 DNA
Catalog number:	KB-COT
Lot number:	See label on the vial
Unit Size:	500 µg
Concentration:	1 mg/ml
Storage buffer:	10 mM Tris-HCl (pH 7.4), 0.3 mM EDTA
Storage conditions:	FISH-grade *cot* can be stored for long term storage at -20°C.

A. Introduction

FISH-grade *cot* is extracted from human placental DNA and subsequently fragmented by sonication, denatured, and re-annealed under conditions that enrich for repetitive DNA sequences (1). FISH-grade *cot* can be used to suppress cross-hybridization (3,4) to human repetitive DNA when human DNA probes are hybridized *in situ (2,3)*.

B. Application

Preparation of probes suppressed with FISH-grade *cot*:

The optimum amount of FISH-grade *cot* required obtaining effective suppression of repetitive DNA sequence depends on the specific application. To establish optimal suppression for certain probes titration of probes using increasing amounts of FISH-grade *cot* or extending the time for pre-hybridization may be required.

C. Protocol

Suggested protocol for *in situ* hybridization:

FISH-grade *cot* can be used in the range of 10-50 fold excess to the probe used. Initial experiments using 25 fold excess are recommended. Additional descriptions of *in situ* hybridization methods using various probes can be found in literature (3-6).

1. Add an excess (10- 50 x) of FISH-grade *cot* to the labelled DNA probe. Add 1/4 volume of 10 M ammonium acetate and 2.5 volumes of ethanol. Place at -80 °C for 30 minutes or at -20 °C overnight.
2. Centrifuge, remove the supernatant carefully, wash with 70% ethanol, and dry the pellet.
3. Dissolve the precipitated probe/FISH-grade cot in 50% formamide / 2 x SSC / 10% dextran sulfate, and vortex extensively.
4. Add the probe/FISH-grade cot mixture to the slide, cover with a glass cover slip. Optional: If the hybridization time is longer than overnight, seal with rubber cement.
5. Denature probe mixture by incubating slides on a hot plate at 80 °C for 5 minutes.
6. Hybridize at 37 °C in a moist chamber.
7. Wash and process the slides using procedures appropriate for the detection method (fluorescent, enzymatic).

D. References

1. Weiner, A.M., et al. (1986) *Ann. Rev. Biochem. 55*, 631.
2. Britten, R.J., et al. (1986) *Methods Enzymol. 29*, 363.
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4. Lengauer, C., et al. (1990) *Hum. Genet. 86*, 1.
5. Lichter P, et al. (1988) *Hum. Genet. 80*, 224.
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