Product information

Version 1.0

Product:	**KREA*cot* DNA**
Description:	**Human C_0t-1 DNA**
Catalog number:	EA-020
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:	KREA*cot* is stable at 4°C for at least 3 months, for long term storage KREA*cot* can be stored at -20°C in a constant temperature freezer.

Introduction:

KREA*cot* DNA is extracted from human placental DNA and subsequently fragmented by sonication, denatured, and re-annealed under conditions that enrich for repetitive DNA sequences (1). KREA*cot* DNA can be used to suppress cross-hybridization (3,4) to human repetitive DNA when human DNA probes are hybridized *in situ (2,3)*. It is also used to suppress cross-hybridization to human repetitive DNA in arrayCGH experiments (4). Furthermore, labelled KREA*cot* DNA serves as an effective hybridization probe for checking cosmids or BACs obtained from human hybrid libraries.

Selected applications:

Preparation of probes suppressed with KREA*cot* :

The optimum amount of KREA*cot* DNA 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 KREA*cot* DNA or extending the time for pre-hybridization may be required.

***In situ* hybridization:**

KREA*cot* DNA 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 the literature (3-6).

1. Add an excess (10- 50 x) of KREA*cot* DNA to the labelled probe DNA. 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. Centrifuge, remove the supernatant carefully, wash with 70% ethanol, and dry the pellet.
2. Dissolve the precipitated probe/ KREA*cot* DNA in 50% formamide, 2X SSC, and 10% dextran sulfate, and vortex extensively.
3. Add the probe/ KREA*cot* DNA mixture to the slide, cover with a glass coverslip.
4. 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 (30% formamide, 2 x SSC).
7. Wash and process the slides using procedures appropriate for the detection method (fluorescent, enzymatic).

ArrayCGH:

KREA*cot* DNA can be used to suppress cross-hybridization to human repetitive DNA in comparative genomic hybridization microarray experiments in combination with the ULS™ arrayCGH Labeling Kits (EA-005, EA-007)

1. Label reference and sample DNA with ULS™-fluorescent dyes according to the manual of the ULS™ arrayCGH Labeling Kit.
2. Pool the labelled reference and sample DNA and add a 12.5 x excess of KREA*cot* DNA
3. Proceed with precipitation and hybridization as described.

Filter hybridization with biotin labelled probes:

1. Label and purify the probe DNA according to the Platinum*Bright*™ Biotin labeling kit manual (GLK-007).
2. Concentrate KREA*cot* DNA (1 mg/ml) by ethanol precipitation to 10 mg/ml. Remove desired amount of KREA*cot* DNA and add 1/4 volume of 10 M ammonium acetate and 2.5 volumes of ethanol. Mix, place at -80 °C for 30 minutes or at -20 °C overnight. Centrifuge,

dry the pellet and resuspend in TE buffer at a concentration of 10 mg/ml

3. Mix in a 1.5 ml tube: 25 to 500 ng labelled DNA (in 100 µl), 5 µl of concentrated KREA*cot* DNA (50 µg), 50 µl of 20X SSC, 25 µl water, and 20 µl of 1% (w/v) SDS.
4. Denature by placing into boiling water for 5 minutes.
5. Transfer to a 65°C water bath and incubate for at least 20 minutes.
6. Add probe/ KREA*cot* DNA mixture to the filter.
7. Incubate and process the filters using procedures appropriate for the detection method.

Labeling KREA*cot* DNA:

KREA*cot* DNA can be labeled with fluorescent dyes or biotin using the Platinum*Bright*™ labeling kits (GLK-001 to GLK-007) following the standard protocol described in the manual.

Note: KREA*cot* DNA purity, DNA size, and concentration are verified by spectrophotometry and agarose gel electrophoresis. Functional validation test have been performed by FISH.
The human-source 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), humanT-cell lymphotropic virus (HTLV-1 and HTLV-2) and *Treponema pallidum*. Handle as if potentially infectious.

References

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Additional literature

1. Marx K.A. et al. (1976) *Biochem. Biophys. Acta* 425(2) p129-47
2. Reid T, et al. (1990) *Hum. Genet.* 85(6) p581-6
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