

Version 1.0

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| <u>Product:</u> | KREAcot DNA |
| <u>Description:</u> | Human C₀t-1 DNA |
| <u>Catalog number:</u> | EA-020 |
| <u>Lot number:</u> | See label on the vial |
| <u>Unit Size:</u> | 500 µg |
| <u>Concentration:</u> | 1 mg/ml |
| <u>Storage buffer:</u> | 10 mM Tris-HCl (pH 7.4), 0.3 mM EDTA |
| <u>Storage conditions:</u> | KREAcot is stable at 4°C for at least 3 months, for long term storage KREAcot can be stored at -20°C in a constant temperature freezer. |

Introduction: KREAcot 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). KREAcot 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 KREAcot DNA serves as an effective hybridization probe for checking cosmids or BACs obtained from human hybrid libraries.

Selected applications:

Preparation of probes suppressed with KREAcot :

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

In situ hybridization:

KREAcot 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 KREAcot 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/ KREAcot DNA in 50% formamide, 2X SSC, and 10% dextran sulfate, and vortex extensively.
3. Add the probe/ KREAcot 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:

KREAcot 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 KREAcot 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 PlatinumBright™ Biotin labeling kit manual (GLK-007).
2. Concentrate KREAcot DNA (1 mg/ml) by ethanol precipitation to 10 mg/ml. Remove desired amount of KREAcot 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 KREAcot 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/ KREAcot DNA mixture to the filter.
7. Incubate and process the filters using procedures appropriate for the detection method.

Labeling KREAcot DNA:

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

Note: KREAcot 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

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.

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
3. Landegent, J.E., et al. (1986) *Hum. Genet.* 77, p366.
4. Raap A.K., et al. (2004) *Biotechniques* 37(1) p130-4

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