For life science research only.
Not for use in diagnostic procedures.

COT Human DNA

from human placenta DNA, enriched for repetitive sequences

Version: 13
Content Version: July 2021

Cat. No. 11 581 074 001	500 µg 500 µl

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	COT Human DNA	▪ Aqueous solution ▪ 1 mg/ml 10 mM Tris-HCl, 1 mM EDTA, pH 7.4	1 vial, 500 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	COT Human DNA	Store at −15 to −25°C. ⚠ ***Repeated freezing and thawing does not deteriorate the product.***

1.3. Additional Equipment and Reagent required

Standard protocol for single-copy probes

- Salmon sperm DNA, sheared to a size of approximately 500 nucleotides, such as DNA, MB grade*
- 3 M sodium acetate, pH 5.0
- Ethanol, 100%, 70%
- Formamide*, deionized
- 20x SSC*
- Dextran sulfate
- Water bath
- Coplin jar
- Humidified chamber

1.4. Application

Use COT Human DNA to suppress cross-hybridization to human repetitive sequences in filter and *in situ* hybridizations and microarrays.

2. How to Use this Product

2.1. Before you Begin

Working Solution

Hybridization buffer: 50% formamide, 2x SSC, 10% dextran sulfate.

2.2. Protocols

In situ hybridization using COT Human DNA

The optimum amount of COT Human DNA required for effective suppression of cross-hybridization to repetitive elements depends on the type and amount of probe DNA. Start with a 50- to 100-fold excess of COT Human DNA compared to the amount of probe DNA. When there is still considerable staining of the chromosomes after *in situ* hybridization caused by repetitive sequences, repeat the experiment with more competitor DNA.

Standard protocol for single-copy probes

The protocol may be modified when using different types of probes.
See section, **Working Solution** *for information on preparing solutions.*

1. Combine 40 to 120 ng of labeled single-copy DNA with 2 to 6 µg of COT Human DNA; add salmon sperm DNA to adjust a total amount of 20 µg DNA.
2. Add 1/10 volume of 3 M sodium acetate and 2 volumes of pre-chilled 100% ethanol; mix and incubate at −70°C for 30 minutes.
3. Centrifuge for 15 minutes at +2 to +8°C; discard supernatant.
4. Wash pellet by adding 400 µl 70% ethanol, centrifuge for 5 minutes, decant supernatant, and lyophilize the pellet.
5. Resuspend the pellet in 20 µl Hybridization buffer by vortexing for several minutes.
6. Denature the DNA mixture by incubating at +75°C for 5 minutes.
7. Incubate the DNA mixture at +37°C for 15 minutes (preannealing).
8. While the DNA mixture is preannealing, denature metaphase chromosomes.
   – Incubate slides prewarmed to approximately +60°C (incubator) in 70% formamide, 2x SSC at +70°C in a Coplin jar for exactly 2 minutes using a water bath.
9. Add the pre-annealed DNA mixture to the denatured chromosomes; apply a coverslip and seal the edges with rubber cement.
10. Transfer the slide to a moist chamber and hybridize overnight at the appropriate temperature, usually +37°C.
11. Wash and process the slide using procedures appropriate for the detection method.

Filter hybridization using COT Human DNA

COT Human DNA can also be used to suppress cross-hybridization to human repetitive DNA during filter hybridization experiments.

1. Mix COT Human DNA in a 100- to 200-fold excess over the labeled probe DNA.

2. Precipitate the mixture and resuspend the pellet in an appropriate hybridization buffer.

3. Denature and pre-anneal as stated in the *in situ* hybridization protocol.
   – The pre-annealed DNA mixture is added to the filter.

3. Results

Typical analysis

In agarose gel electrophoresis, the length distribution of the COT Human DNA fragments shows a maximum in the range of 50 to 300 nucleotides. COT Human DNA suppresses cross-hybridization to human repetitive sequences in filter and *in situ* hybridizations.

4. Additional Information on this Product

4.1. Test Principle

The COT fraction of human genomic DNA consists largely of rapidly annealing repetitive elements. These interspersed repetitive sequences (IRS) such as SINEs (small interspersed repetitive elements, for example, Alu-elements) and LINEs (large interspersed repetitive elements, for example, L1-elements) are distributed ubiquitously throughout the genome.
COT Human DNA is used in chromosomal *in situ* suppression (CISS) hybridization. IRS present in a probe, such as cosmids, YACs, or chromosome painting probes, result in nonspecific hybridization signals distributed over the whole chromosome or genome.

- To enable specific hybridization of the probe to the chromosomal target site, such as single-copy sequences or low-copy repeats, the probe is denatured together with an excess of unlabeled COT Human DNA as competitor.
- Subsequent preannealing allows rapid hybridization of the repetitive probe elements with the excess repeats of the COT Human DNA, while most of the specific probe sequences remain single-stranded and thus enable hybridization to their chromosomal targets.

Preparation

COT Human DNA is prepared from human placental DNA by shearing, denaturing, and reannealing under conditions that enrich these repetitive elements.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
DNA, MB-grade	500 mg, 50 ml, 10 mg/ml	11 467 140 001
Formamide	500 ml	11 814 320 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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