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

COT Human DNA, Fluorometric Grade

from human placenta DNA, enriched for repetitive sequences

Version: 06
Content Version: July 2021

Cat. No. 05 480 647 001 1mg
1 ml

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	COT Human DNA, Fluorometric Grade	▪ Aqueous solution in 10 mM Tris-HCl, 1 mM EDTA, pH 7.2. ▪ 1 to 1.5 mg/ml concentration.	1 vial, 1 mg, 1 ml

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, Fluorometric Grade	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

For DNA microarray hybridization

- 20x SSPE
- Formamide*
- Poly(A)
- SDS* (10%)
- Labeled target
- Water bath
- Hybridization chamber

1.4. Application

Use COT Human DNA, Fluorometric Grade 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

Preparation of hybridization mix

- 2.5 to 5x SSPE buffer
- 0.1 to 0.25% SDS*
- 50% formamide*
- 2 to 6 µg of poly(A)
- Labeled target

Volumes based on hybridization area

- 24 × 24 mm cover slip: Approximately 16 µl
- 24 × 40 mm cover slip: Approximately 24 µl
- 24 × 60 mm cover slip: Approximately 32 µl

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 COT Human DNA.

DNA microarray hybridization

See section, **Working Solution** *for information on preparing solutions.*

1. Add 20 to 50 µg of COT Human DNA to the prepared Hybridization mix.

2. Heat the Hybridization mix at +100°C for 2 minutes.

3. Cool the Hybridization mix at +30°C in a water bath for 30 seconds.

4. Transfer the appropriate amount of hybridization mix onto the DNA microarray.
   – Incubate for 14 to 16 hours at +42°C in a hybridization chamber or commercially available hybridization instrument.

The protocol may be modified when using different types of probes.

Filter hybridization using COT Human DNA

COT Human DNA can 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.
3. Resuspend the pellet in an appropriate hybridization buffer.
4. 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.

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 (male gender) by shearing, denaturing, and reannealing under conditions that enrich these repetitive elements.

4.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

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		
Formamide	500 ml	11 814 320 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001

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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