SPoT-Light™ Tissue Pretreatment Kit GPR

Catalog Number 008401

Pub. No. MAN0004480 Rev. D

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

For use in heat pretreatment and enzyme digestion of formalin-fixed, paraffin-embedded (FFPE) tissue prior to chromogenic in situ hybridization (CISH) detection.

Contents and storage

Items	Cat. No.	Quantity	Storage[1]
Heat Pretreatment Solution, pH 7.0	100200104	1 L	2–8°C
Enzyme Reagent	100200105	10 mL	

[1] Do not use after the expiration date stamped on the container.

Procedural guidelines

- Do not allow tissue sections to dehydrate.
- Probe denaturation caused by a lower than recommended temperature can result in a weak or absent CISH signal.
- Hybridization done for shorter time periods or stringent washes done at higher temperatures than recommended can result in a decrease or complete loss of the CISH signal.

Protocol

Prepare the tissue

1. Treat slides with an adhesive before mounting tissue sections.
2. Deparaffinize slides and dehydrate tissue sections.
3. Perform heat pretreatment and enzyme digestion as recommended in the instructions that are provided with the ISH probe.

   Note: If probe instructions are not available, proceed to “Pretreat the slides” on page 1, then “Digest with Enzyme Reagent” on page 2.

Pretreat the slides

1. Heat 500 mL of SPoT-Light™ Heat Pretreatment solution in a beaker on a hot plate until it is boiling or 98–100°C.
2. Boil the slides for 15 minutes.

   Note: Different incubation times may be required depending on tissue fixation. A 15-minute incubation is a recommended starting point.
3. Wash in PBS or deionized water at room temperature for 3 minutes.
4. Discard the solution and wash in fresh PBS or deionized water at room temperature for 3 minutes.

Digest with Enzyme Reagent

- Depending on the fixative used, an incubation time of 5–15 minutes may be required. Excessive digestion causes loss of nuclei and chromosome structure.
- For most breast tissue, 10 minutes of enzyme digestion at room temperature produces the best CISH results.
- Ensure to prewarm the Enzyme Reagent to room temperature prior to adding the tissue section.

1. Cover the tissue with 100–200 µL of Enzyme Reagent at room temperature for 10 minutes.
2. Wash in PBS or deionized water at room temperature for 3 minutes.
3. Discard the solution and wash in fresh PBS or deionized water at room temperature for 3 minutes.
4. Dehydrate the slides in a series of 70%, 85%, 95%, and 100% ethanol for 2 minutes each at room temperature.
5. Air dry the slides, then proceed to denaturation and hybridization.

Evaluate the specimen

After completing the protocol, evaluate the enzyme pretreatment of the specimen.

Observation	Cause
Nuclei are not counter stained and there is an absent or very weak CISH signal.	Possible nuclear loss as a result of excessive digestion with Enzyme Reagent.
Nuclei are strongly counter-stained but a CISH signal is absent in the nuclei.	Possible under-digestion with Enzyme Reagent.

Note: If optimal results are not attained, enzyme pretreatment can be performed at 37°C for 3 minutes.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
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  - Training for many applications and instruments
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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0004480 D

Revision	Date	Description
D	20 March 2025	• Added new item numbers in Contents and Storage. • Minor updates were made throughout for consistency of style and terminology. • Version format was changed in conformance with internal document control procedures.
3.0	23 March 2021	Change in regulatory classification to GPR. Updated to the latest Thermo Fisher Scientific template, with associated changes to logos, trademarks, and format.
2.01	14 May 2021	Baseline for revision.