A Sysmex Group Company

CytoCell

Instructions For Use
REF: LPS 100

Tissue Pretreatment Kit

PRODUCT FOR GENERAL LABORATORY USE

FOR LABORATORY USE

PROFESSIONAL USE ONLY

ogt.com/CytoCell

Further information and other languages available at ogt.com/CytoCell

Intended Purpose
For use in heat pretreatment and enzyme digestion of Formalin-Fixed, Paraffin Embedded (FFPE) tissue prior to Fluorescence *in situ* Hybridisation (FISH) or Chromogenic *in situ* Hybridisation (CISH) detection.
This product is intended for general laboratory use or use in a Laboratory Developed Test (LDT). If used as part of an LDT, it is the responsibility of the laboratory developing the test to validate the test before use in a clinical setting.

Materials Provided
Reagent 1 (LPS 100A):
One Litre of Heat Pretreatment Solution, pH 7.0 (Ready to Use)
Reagent 2 (LPS 100B):
One 10ml bottle of Enzyme Reagent (contains Pepsin, Ready to Use)

Warnings and Precautions
1. Product for General Laboratory Use. For laboratory professional use only.
2. USA: General Purpose Reagent (GPR). For Laboratory Use.
3. Heat Pretreatment Solution: Handle with care; wear gloves and a lab coat.
4. Enzyme Reagent: Contains Pepsin. Handle with care; wear gloves and a lab coat.
5. Do not use if the kit contents are damaged or compromised in any way.
6. Follow local disposal regulations for your location along with recommendations in the Safety Data Sheet to determine the safe disposal of this product. This also applies to damaged kit contents.
7. Dispose of all used reagents and any other contaminated disposable materials following procedures for infectious or potentially infectious waste. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.
8. Failure to adhere to the outlined protocol may affect the performance of the reagents.

Temperature Definitions
- 4°C / 2-8°C / In a Refrigerator +2°C to +8°C
- 37°C: +37°C ± 1°C
- Room Temperature (RT): +15°C to +25°C

Storage and Handling
Store at 2-8°C in a refrigerator until the expiry date indicated on the label.

Materials and ancillaries recommended but not provided with LPS 100
- Porcelain wash jars (PCN 009)
- Hotplate (capable of reaching & holding 37°C for at least 30mins)
- Waterbath (capable of reaching & holding 98-100°C for at least 20-30mins)
- Fumehood/safety cabinet (for deparaffinisation step)
- Mercury thermometer
- Slide surface thermometer (PCN 002)
- Glass slides (Superfrost or similar, positively charged)
- Glass coverslips (digestion)

Stability
There are no obvious signs to indicate instability of these reagents. They have been quality controlled to assure consistent and reliable performance. Do not use after the expiration date stamped on container. With proper storage there is no significant loss of performance. If the reagents are stored under any conditions other than those specified, conditions must be validated by the user.

Tissue Preparation
For optimal results, (+) charged slides are advised. This pre-treatment kit is intended for use after FFPE slides have been de-paraffinised and rehydrated according to standard cytogenetic procedures. Perform heat pretreatment and enzyme digestion as recommended in the instructions that come with the ISH probe. The following procedure may be used if probe instructions are unavailable.

Heat Pretreatment
1. Heat 50ml Tissue Pretreatment Solution (Reagent 1) in a porcelain wash jar or coplin jar immersed in a waterbath until it is either boiling or 98 - 100°C. Boil slides for 30 minutes (Note: different incubation times may be required depending on tissue fixation. A 30-minute incubation is a recommended starting point). Note: for safety we recommend the use of double gloves and tweezers to handle the slides. Porcelain wash jars (PCN 009) provide better temperature stability than traditional glass coplin jars, and are less likely to break.

2. Wash in dH_2O at room temperature (RT) for 2 x 3 minutes.

Enzyme Digestion
Enzyme should be removed from the fridge and brought to room temperature prior to application.
1. Cover tissue with Enzyme Reagent* (Reagent 2) for 10 minutes^ at 37°C on a hotplate or hybridiser. *the amount of enzyme reagent needed may vary between specimens, but should be sufficient to cover the entire tissue section. ^ depending on tissue fixative used, different incubation times may be required. Excessive digestion will cause loss of nuclei and chromosome structure. Please refer to Troubleshooting section for details.
2. Return the enzyme reagent to the fridge to maintain stability.

Note: ensure that the tissue section does not dry out during digestion. More enzyme reagent may need to be added to prevent this. Please contact the support team at techsupport@cytocell.com or see the FISH 'n' Tips section of the CytoCell website for advice on how to achieve optimal protocol for your laboratory's needs.
3. Wash in dH_2O at RT for 3 x 2 minutes.
4. Dehydrate slides in a series of 70%, 85%, 100% and 100% ethanol for 2 minutes each at room temperature and allow to air dry.

FISH Protocol
The slides are now ready for probe application, please refer to the specific probe IFU for details or consult the protocol of your own validated LDT.

Troubleshooting
1. Throughout the entire procedure, unless otherwise indicated, it is important that the tissue section does not dehydrate.
2. **Heat Pretreatment (A critical step for successful performance):** The specimen must be boiled or heated above 98°C for 30 minutes in Heat Pretreatment Solution. Local conditions including altitude, humidity etc. may affect the boiling point of the solution.
3. **Enzyme Digestion (The most critical step for successful performance):** Different enzyme incubation times (5 - 45 minutes) may be required, depending on tissue type and fixation method. **For most breast tissues, 10 minutes enzyme digestion at 37°C will produce the best results. Be sure to pre-warm the Enzyme Pretreatment Reagent to RT prior to adding to the tissue section.** Enzyme pretreatment of the specimen should be evaluated immediately at the completion of the hybridisation protocol. If nuclei are not DAPI counterstained and there is an absent or very weak signal, this may be due to nuclear loss as the result of excessive digestion. If nuclei are strongly counterstained but a signal is absent in the nuclei, this may be due to under-digestion during the pepsin pretreatment.
4. Probe denaturation at a temperature lower than recommended by the protocol may result in a weak or absent signal.
5. Hybridisation performed for shorter time periods, or stringent washes performed at higher temperatures, than recommended by the protocol may produce a decrease in or complete loss of the signal.

Recommended timings of common Tissue Types:

Tissue type	Pre-treatment time (mins)	Enzyme digestion (mins)
Breast	30	10 – 40
Lung	25	15 – 20
Ovary	20	10
Kidney	20	20 – 25
Colon	30	20
Schwann cells (nerve tissue)	30	15
Brain	30	15 – 18

Optimal pretreatment and digestion times vary. More troubleshooting help, as well as FISH n' Tips advice and technical guides can be found on the support pages of the OGT website.

Additional Information
For additional product information please contact the CytoCell Technical Support Department.
T: +44 (0)1223 294048
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W: www.ogt.com

References

1. Wilkinson, D.G., *In Situ Hybridization, a Practical Approach*. 2nd ed., Oxford university Press, Oxford, (1998).
2. Polak, J.M. and McGee, J. *In Situ Hybridization: Principles and Practice.* Oxford University Press, Oxford, UK, (1998).
3. Verma, R.S and Babu, A. *Human Chromosomes: Principles and Techniques.* 2nd ed.,Health Professions Division, New York, (1995).
4. Leitch, A.R. et al. *In Situ Hybridization-A Practical Guide: Royal Microscopy Society Microscopy Handbooks.* Vol 27, Bios Scientific Publishers, Oxford, UK, (1994).

Symbols Glossary

EN ISO 15223-1:2021 - "Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements" (© International Organization for Standardization)		
Symbol	**Title**	**Reference Number(s)**
	**en:** Manufacturer	5.1.1
	**en:** Use-by date	5.1.4
	**en**: Batch code	5.1.5
	**en:** Catalogue number	5.1.6
	**en:** Temperature limit	5.3.7
	**en:** Consult instructions for use	5.4.3
 ogt.com/CytoCell	**en:** Consult electronic instructions for use	5.4.3
	**en:** Caution	5.4.4
EDMA symbols for IVD reagents and components, October 2009 revision		
Symbol	**Title**	**Reference Number(s)**
	**en:** Contents (or contains)	N/A

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