KI-60006 FISH Digestion Kit

Older or difficult samples or slides with cytoplasmic background

RUO - Research Use Only
Not for use in diagnostic procedures

Instructions for use for KI-60006 FISH Digestion Kit in combination with Kreatech™ fluorescent labeled DNA probes

Fluorescent in situ hybridization (FISH) identifies or labels target genomic sequences so that their location can be studied. DNA sequences from appropriate chromosome specific probes are first labeled with reporter molecules. The labeled FISH probe is then hybridized to the metaphase chromosomes or interphase nuclei on a slide. After washing, the specimen is screened for the reporter molecules by fluorescence microscopy.

Kreatech™ probes do not contain Cot-1 DNA. Hybridization efficiency is therefore increased and background, due to unspecific binding, is highly reduced.

For use on **metaphase** and **interphase cells** from **peripheral blood cultures** or direct preparations prepared by **standard cytogenetic methods** see: The ACT cytogenetics laboratory manual. 3rd ed. New York: Raven Press; 1996.

This pretreatment kit is specifically developed to obtain optimal results in case older samples, slides with cytoplasmic background, or difficult samples are used or expected.

Note: Freshly prepared cytological samples
It is advised to use KI-60005 FISH reagent kit in case freshly prepared cytological samples are used.

For paraffin embedded tissues it is recommended to use pretreatment kits KI-60004 or KI-60007.

For more info consult our website: www.LeicaBiosystems.com.

Pretreatment:

1. Pretreat dry sample slide in 2 x SSC (LK-104B), at 37°C for 2 minutes (min).
2. Drop approximately 200 µl of Pepsin Solution (LK-101B) on the cells and incubate at room temperature the slides 30 seconds - 10 min (depending on sample material, fixation time and storage conditions).
3. Wash slide for 3 min in 1 x PBS at room temperature.
4. Post-fix in 1% buffered formaldehyde in 1x PBS / 20 mM $MgCl_2$ for 10 min at room temperature.
5. Wash slide for 3 min in 1 x PBS at room temperature.
6. Dehydrate in 70%, 85% and 100% ethanol for 1 min each. Air-dry at room temperature.
7. Proceed with Probe preparation.

Note: Check protein digestion and pretreatment by applying 15 µl DAPI counterstain and evaluate slides using a fluorescence microscope equipped with a DAPI filter. Remove cover slip and soak tissue in 2 x SSC for 2 min and prolong protein digestion if sample is not sufficiently digested. Use a fresh sample and reduce protein digestion time if the sample is over-digested.

Probe preparation:

Kreatech™ FISH probes are supplied Ready to Use (RtU).
SE, ST, and WC Kreatech™ FISH probes are provided 5 x concentrated and must be diluted as follows: 2 µl 5 x conc. Probe in 8 µl FISH Hybridization Buffer (FHB or WCB, supplied with probes). To combine several 5 x conc. probes, replace FISH Hybridization Buffer (FHB or WCB) by 2 µl for each probe added.

Co-denaturation:

Apply 10 µl of probe or probe-mix per 22 x 22 mm field. Cover with glass cover slip and seal with Fixogum or rubber cement. Denature sample and probe on a Thermo*brite* (TS-01/02) or on a hot plate at 75 ±1 °C for 5-10 min. Continue with hybridization.

Hybridization:

Incubate overnight at 37 ±1 °C on a Thermo*brite* (TS01/02) or in a humidified chamber.

Post-Hybridization Wash:

1. Pre-warm Wash Buffer I (0.4 x SSC / 0.3% Igepal) (LK-102) to 72 °C
2. Remove rubber cement.

3. Place up to 14 slides in 200 ml of Wash Buffer II (2 x SSC / 0.1% Igepal) (LK-103), incubate for 2 min at RT. Slide off coverclips. Re-use only once for a total of 28 slides.
4. Place up to 14 slides in 200 ml of pre-warmed Wash Buffer I (0.4 x SSC / 0.3% Igepal) (LK-102), incubate for 2 min at 72 °C (± 1 °C) without agitation. Re-use only once for a total of 28 slides.
5. Place up to 14 slides in 200 ml of fresh Wash Buffer II (2 x SSC / 0.1% Igepal) (LK-103), incubate for 1 min at RT without agitation. Re-use only once for a total of 28 slides.
6. Dehydrate in fresh 70%, 85% and 100% ethanol, incubate for 1 min each at RT. Air dry at RT and proceed to counterstaining.

Counterstaining:

Apply 15 µl DAPI counterstain (DAPI/Antifade 0.1 µg/ml) (LK-095B) and apply glass cover slip. Proceed with microscopy.

Procedural recommendations:

Temperature and buffer concentration (stringency) of hybridization and washing are important, as lower stringency can result in non-specific binding of the probe to other sequences and higher stringency can result in a lack of signal. Incomplete denaturation of target DNA can result in lack of signal.

Material provided:

LK-104B	2 x SSC solution
LK-102B	Wash Buffer I
LK-103B	Wash Buffer II
LK-101B	Pepsin Solution
LK-095B	DAPI Counterstain (0.1 µg/ml)
LK-097B	Counterstain diluent

Recommendations for Fluorescence Microscopy:

For optimal visualization use a well maintained and regularly calibrated microscope equipped with a 100 W mercury lamp and a 63x or 100x fluorescent objective. Triple band-pass filters (DAPI/FITC/Texas Red or DAPI/FITC/Rhodamine) are used to view multiple colors, single band-pass filters are used for individual color visualization.

Suitable excitation and emission range for Kreatech™ fluorophores:
Platinum*Bright*™415 Ex 429 ±20 nm Em 470 ±30 nm
Platinum*Bright*™495 Ex 495 ±20 nm Em 525 ±30 nm
Platinum*Bright*™ 550 Ex 546 ±12 nm Em 580 ±30 nm

Material required, but not supplied:

- Ethanol 100%, 85% and 70%
- Fixogum (LK-071A) or rubber cement
- Hot plate with accurate temperature control up to 75 °C or Thermo*Brite*™ (TS01/TS02)
- Incubator with accurate temperature control at 37 °C
- Waterbath with accurate temperature range of 37-75 °C
- Plastic or glass coplin jars
- Variable micropipettes (1 µl - 200 µl)
- Fluorescence microscope equipped with suitable filters (see recommendations for Fluorescence Microscopy).

Warnings and Precautions:

1. For professional use only. In case of emergencies check SDS sheets for safety information.
2. FISH probes and hybridization buffers contain formamide which is a teratogen; do not inhale or allow skin contact. Wear gloves and a lab coat when handling FISH probes and DAPI counterstain.
3. All materials should be disposed of according to your institution's guidelines for hospital waste disposal.

OSHA HazCom Standard (2012)

Code #	Description	Signal Word	Pictogram	Hazard Statements
LK-095B	DAPI/Antifade 0.1 µg/ml (DAPI Counterstain)	Warning		H315, H319, H335
LK-097B	Antifade (Counterstain diluent)	Warning		H315, H319, H335
LK-101B	Pepsin Solution	N.A.	N.A.	N.A.
LK-102B	0.4 x SSC / 0.3% Igepal (Wash Buffer I)	N.A.	N.A.	N.A.
LK-103B	2 x SSC / 0.1% Igepal (Wash Buffer II)	N.A.	N.A.	N.A.
LK-104B	2 x SSC	N.A.	N.A.	N.A.

(*Please note that LK-102 and LK-103 products containing the same number but different letters are identical except for the volume.
A= 100 ml, B=250 ml)

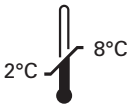
Technical support

Technical support is available at
www.leicabiosystems.com or toll free at 800-248-0123 or via e-mail:
kreatech-support@leicabiosystems.com.

Customer service

Kreatech probes may be ordered through Leica Customer Service toll free at 800-248-0123
or order via e-mail: purchase.orders@leica-microsystems.com.

LeicaBiosystems.com



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IfU KI-60006 FISH digestion kit_D2.4 · Published: March 2018 ·