KBI-60006 FISH Digestion Kit

Older or difficult samples or slides with cytoplasmic background

Instructions for use for KBI-60006 FISH Digestion Kit in combination with Kreatech™ FISH 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 DNA 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.

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. 2nd ed. New York: Raven Press; 1991.

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 KBI-60005 FISH reagent kit in case freshly prepared cytological samples are used.

For paraffin embedded tissues it is recommended to use pretreatment kits KBI-60004 or KBI-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 (RT) 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 RT.
4. Post-fix in 1% buffered formaldehyde in 1x PBS / 20 mM MgCl2 for 10 min at RT.
5. Wash slide for 3 min in 1 x PBS at RT.
6. Dehydrate in 70%, 85% and 100% ethanol for 1 min each. Air-dry. 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) at 75 ±1 °C for 5 - 10 min. Continue with hybridization.

Hybridization:

Incubate overnight at 37 ±1 °C in a Thermo*brite* (TS-01/02) 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 0.1µg/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.

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 415 ±20 nm Em 475 ±30 nm
Platinum*Bright*™495 Ex 495 ±20 nm Em 525 ±30 nm
Platinum*Bright*™ 550 Ex 546 ±12 nm Em 580 ±30 nm

Warnings and Precautions:

1. **For *in vitro* diagnostic use.** For professional use only. In case of emergencies check SDS sheets for safety information.
2. DNA 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 DNA probes and DAPI counterstain.
3. All materials should be disposed of according to your institution's guidelines for hospital waste disposal.

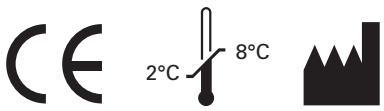
Labelling According Regulation (EC) No 1272/2008

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.

Material provided:

Code #	Description	Volume
LK-095B	DAPI/Antifade 0.1 µg/ml (DAPI counterstain)	0.5 ml
LK-097B	Antifade (Counterstain diluent)	0.5 ml
LK-101B	Pepsin Solution	5 ml
LK-102B	0.4 x SSC / 0.3% Igepal (Wash Buffer I)	250 ml
LK-103B	2 x SSC / 0.1% Igepal (Wash Buffer II)	2 x 250 ml
LK-104B	2 x SSC	250 ml

(*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)



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