KBI-60007 Tissue Digestion Kit I

For conventional paraffin-embedded tissues

Instructions for use for the KBI-60007 Tissue Digestion Kit I 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.

This pretreatment kit is specifically developed to obtain optimal results on conventional paraffin-embedded tissues.

Note: Difficult paraffin-embedded tissues
The use of the KBI-60004 Tissue Digestion Kit II is recommended for use with heavily cross-linked or difficult samples (e.g. longer fixation or prolonged storage).

For metaphase and interphase cells or other cytological samples it is recommended to use pretreatment kit KBI-60005 or KBI-60006.

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

Pretreatment:

Mount 4-6 µm formalin-fixed paraffin-embedded tissue sections on positively charged slides (e.g. aminoalkylsilane)

1. Bake mounted slides for 2 hours at 80 °C or 16 hours at 56 °C.
2. De-paraffinize warm slides by soaking in xylene or xylene substitute for two times 10 minutes (min).
3. Re-hydrate by soaking in 100%, 85% and 70% ethanol for 3 min each.
4. Wash with dH_2O for 3 min at room temperature (RT).
5. Place slides in Pretreatment Solution A (LK-110B) at 96-98°C for 15 min.
6. Rinse twice with dH_2O for 2 min at RT.
7. Cover paraffin section with approximately 200 µl **Pepsin Solution (LK-101B) and incubate at RT for 5 - 50 min** (Time depending on tissue fixation and tissue type. E.g. most breast cancer tissue needs 5 - 15 min digestion; colon tissue 20-30 min).
8. Wash in dH_2O for 1 min.
9. Wash in 2 x SSC (LK-104B) for 5 min at RT.
10. Dehydrate slides by soaking in 70%, 85%, and 100% ethanol for 1 min each time. 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 overdigested.

Probe preparation:

Kreatech™ FISH probes for paraffin embedded tissue are supplied Ready to Use (RtU) unless specified otherwise in the product documentation. Consult label on vial and specific probe pack insert for dilution specifics.

Co-denaturation:

Apply 10 µl of probe 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 80 ±1 °C for 5 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 1 µg/ml) (LK-096B) and apply glass cover slip. Proceed with microscopy.

Procedural recommendations:

The wash concentrations (stringency), pH and temperature are important, as low 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-096B	DAPI/Antifade 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.
LK-110B	0.01 M sodium citrate (Pretreatment Solution A)	N.A.	N.A.	N.A.

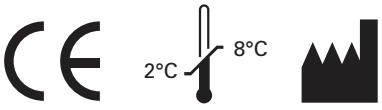
Material provided:

Code #	Description	Volume
LK-096B	DAPI/Antifade 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
LK-110B	0.01 M sodium citrate (Pretreatment Solution A)	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)

Material required, but not supplied:

- Xylene
- Ethanol 100%, 85% and 70%
- Fixogum (LK-071A) or rubber cement
- Hot plate with accurate temperature control up to 80 °C or ThermoBrite™ (TS01/TS02)
- Incubator with a range of 37-56 °C
- Water bath with accurate temperature range of 37- 98 °C
- Plastic or glass coplin jars
- Variable micropipettes (1 µl - 200 µl)
- Fluorescence microscope equipped with suitable filters (see recommendations for Fluorescence Microscopy).



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