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 POSEIDON™ Repeat-Free™ 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 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.

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

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.kreatech.com

Pretreatment:

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

1. Bake mounted slides for 2-16 hours at 56 ºC.
2. De-paraffinize warm slides by soaking in xylene for two times 10 minutes (min). Re-hydrate by soaking in 100%, 85% and 70% ethanol for 3 min each. Wash with dH_2O for 3 min at room temperature.
3. Place slides in Pretreatment Solution A (LK-110B) at 96-98 ºC for 15 min. Rinse twice with dH_2O for 2 min at room temperature.
4. Cover paraffin section with approximately 200 µl **Pepsin Solution (LK-101B) and incubate at room temperature 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). Wash in dH_2O for 1 min and in 2 x SSC (LK-104B) for 5 min at room temperature.
5. 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:

POSEIDON Repeat-Free probes for paraffin embedded tissue are supplied Ready to Use (RtU).

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 hot plate at 80 ±1 ℃ for 5 min. Continue with hybridization.

Note: Probes have been qualified on semi-automated hybridization machines (e.g. ThermoBrite™)

Hybridization:

Incubate overnight at 37 ±1 ℃ in a humidified chamber.

Post-Hybridization Wash:

1. Remove rubber cement seal.
2. Wash slides in Wash Buffer II (LK-103B) for 2 min at RT to slide off cover slip.
3. Wash slides in Wash Buffer I (LK-102B) for 2 min at 72 ±1 ℃ without agitation. Do not place more than 5 slides in the wash at one time. If more than 5 slides are to be washed, verify that the temperature on the wash solution is 72 ℃ ±1 ℃

Note: Do not remove the cover slips from several slides before placing any of the slides in the Wash bath. Begin timing the 2 min incubation when the last slide has been added to the wash.

4. Wash slides in Wash Buffer II (LK-103B) for 1 min at room temperature without agitation.
5. Dehydrate in 70%, 85% and 100% ethanol for 1 min each.
6. Air-dry at room temperature.
7. Proceed to Counterstaining.

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Counterstaining:

Apply 15 µl DAPI counterstain (LK-096B) 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-110B	Pretreatment Solution A
LK-101B	Pepsin Solution
LK-096B	DAPI counterstain (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 Poseidon fluorophores:
PlatinumBright 415 Ex 415 ±20 nm Em 475 ±30 nm
PlatinumBright 495 Ex 495 ±20 nm Em 525 ±30 nm
PlatinumBright 550 Ex 546 ±12 nm Em 580 ±30 nm

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)
- incubator with accurate temperature between 37 °C and 56 °C
- water bath with accurate temperature between 37 °C and 98 °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 in vitro use only. For professional use only.** In case of emergencies check MSDS 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.

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