KBI-60005 FISH Reagent Kit
For freshly prepared samples

Instructions for use for KBI-60005 FISH Reagent Kit
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.

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 on FRESHLY prepared cytological samples.

Note: background or difficult samples
It is advised to use the **KBI-60006 FISH Digestion kit** in case older samples, slides with cytoplasmic background or difficult samples are used or expected.

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

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

Pretreatment:
Pretreat freshly prepared sample slides in 2 x SSC / 0.5% Igepal (LK-105B) at 37 ℃ for 15 minutes (min). Dehydrate in 70%, 85% and 100% ethanol for 1 minute each. Air-dry at room temperature. Proceed with Probe preparation.

Probe preparation:
ON, PN, and MD POSEIDON Repeat-Free probes are supplied Ready to Use (RtU).
SE, ST, and WC POSEIDON Repeat-Free 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) with 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 hot plate at 75 ±1 ℃ for 5-10 min. Continue with hybridization.

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

Hybridization:
Incubate overnight at 37 ±1 ºC 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 of the wash solution is 72 ±1 ℃

Note: Do not remove the coverslips 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.

Counterstaining:
Apply 15 µl DAPI counterstain (LK-095B) and apply glass cover slip. Proceed with microscopy.

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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-105B	2 x SSC / 0.5% Igepal
LK-102B	wash buffer I
LK-103B	wash buffer II
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 Poseidon 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

Material required, but not supplied:
- ethanol 100%, 85% and 70%
- Fixogum (LK-071A) or rubber cement
- hot plate (with accurate temperature control up to 80 ℃)
- incubator at 37 ℃
- water bath with accurate temperature between 37 ℃ and 72 ℃
- 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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