POSEIDON PROBES

Instructions for use

Using Poseidon 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 labeled with reporter molecules. Subsequently the labeled DNA probe is hybridized to 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.

Pretreatment:
Pre-treat fresh prepared sample slides in 2 x SSC, 0.5% Igepal, pH 7.0 at 37℃ for 15 minutes. Dehydra te in 70%, 85% and 100% ethanol for 1 minute each. Air-dry at room temperature. Proceed with the denaturation step.

Or

Optional Pretreatment: (*for slides with cytoplasmic background or difficult samples)*

1. Pre-treat dry sample slide in 2 x SSC, pH 7.0 at 37℃ for 2 minutes.
2. Incubate the slides 5 - 15 minutes (depending on sample material) in 0.005% Pepsin solution in 0.01 M HCl at 37℃.
3. Wash slide for 3 min in 1 x PBS at room temperature.
4. Post-fix in 1% buffered formaldehyde in 1 x 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 minute each. Air-dry at room temperature.
7. Proceed with the denaturation step.

Probe preparation:
POSEIDON probes are supplied Ready to Use (RtU).

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℃ for 5-10 minutes. Continue with hybridization.

Note: Probes have been qualified on half-automated hybridization machines (e.g. HYBrite™, Thermobrite™)

Or

Separate slide denaturation:

1. Denature slide in 70% Formamide/ 2X SSC, pH 7.0 at 72ºC (±1ºC) for 2 minutes.
2. Dehydrate in ice cold (-20ºC) 70%, 85%, and 100% ethanol for 2 minutes each. Air-dry.
3. Denature probe mix at 90℃ for 10 minutes.
4. Apply probe to denatured slide, cover with glass cover-slip, seal with rubber cement and continue with hybridization.

Hybridization:
Incubate overnight at 37ºC in a humidified chamber.

Post-Hybridization Wash:

1. Remove rubber cement, slide off cover-slips.
2. Wash slides in 1 x Wash Buffer II (2 x SSC/ 0.1% Igepal) for 2 minutes at RT (only needed when cover-slips do not slide off easily).
3. Wash slides in 1 x Post-Wash Buffer I (0.4 x SSC/ 0.3% Igepal) for 2 minutes at 72℃ (±1℃) without agitation.
4. Wash slides in 1 x Wash-Buffer II (2 x SSC/ 0.1% Igepal) for 1 minute at RT without agitation.
5. Dehydrate in 70%, 85% and 100% ethanol for1 minute each.
6. Air-dry at room temperature.
7. Proceed to Counterstaining.

Counterstaining:
Apply 15 µL Dark Blue Antifade 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 lack of signal. Incomplete denaturation of target DNA can result in lack of signal.

Material provided:
5 test format RtU: 50 µL of probe.
10 test format RtU: 100 µL of probe.

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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 colours, single band-pass filters are used for individual colour 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:

- 1% buffered Formaldehyde/1 x PBS/20mM $MgCl_2$
- PBS
- Formamide
- 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 at 72℃ / 37°C
- Plastic or glass coplin jars
- Variable micropipettes (1 µL - 200 µL)
- Fluorescence microscope equipped with suitable filters (see recommendations for Fluorescence Microscopy).

Material required and not supplied, but available in the POSEIDON FISH kit (KBI-60002):

- 2 x SSC
- Igepal
- Post-Wash-Buffer I (0.4 x SSC / 0.3% Igepal)
- Wash-Buffer II (2 x SSC / 0.1% Igepal)

Or

POSEIDON FISH & Digestion kit (KBI-60003):

- Pepsin
- Igepal
- 0.01 M HCL
- 2 x SSC
- Post-Wash-Buffer I (0.4 x SSC / 0.3% Igepal)
- Wash-Buffer II (2 x SSC / 0.1% Igepal)

Warnings and Precautions:

1. **For in vitro use only.** For professional use only. In case of emergencies check MSDS for safety information.
2. DNA probes and hybridization buffers contain formamide which is teratogenic; do not breathe or allow skin contact. Wear gloves and a lab coat when handling DNA probes and Light Blue / Dark Blue Antifade counterstain. Upon disposal, flush with a large volume of water.
3. All materials should be disposed of according to your institution's guidelines for hospital waste disposal.

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KREATECH
Diagnostics
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2°C 8°C long term storage