

POSEIDON Repeat-Free™ PROBES



Instructions for use

Using Repeat-Free™ 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 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.

Repeat-Free Poseidon 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.

Pretreatment:

Pre-treat fresh prepared sample slides in 2 x SSC, 0,5 % Igepal, pH 7.0 at 37°C for 15 minutes. Dehydrate in 70%, 85% and 100% ethanol for 1 minute each. Air-dry at room temperature. Proceed with [Probe preparation](#).

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°C 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°C.
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 MgCl₂ 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 [Probe preparation](#).

Probe preparation:

ON, PN, and MD POSEIDON probes are supplied Ready to Use (RtU).

SE, ST, and WC POSEIDON probes are provided at 5X concentration and must be diluted as following: 2 µl 5x conc. Probe in 8 µl FISH Hybridization Buffer (FHB or WHB, supplied with probes). To combine several 5x conc. Probes replace FISH Hybridization Buffer (FHB or WHB) by additional 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°C 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, Optional:

1. Denature slide in 70% Formamide/ 2X SSC, pH 7.0 at 72°C (±1°C) for 2 minutes.
2. Dehydrate in ice cold (-20°C) 70%, 85%, and 100% ethanol for 2 minutes each. Air-dry.
3. Denature probe mix at 90°C 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 37°C in a humidified chamber.

Post-Hybridization Wash:

1. Remove rubber cement, slide off cover-slips.
2. Wash slides in 1 x Post-Wash Buffer II (2 x SSC/ 0.1% Igepal) for 2 minutes at RT.
3. Wash slides in 1 x Post-Wash Buffer I (0.4 x SSC/ 0.3% Igepal) for 2 minutes at 72°C (±1°C) 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 for 1 minute each.
6. Air-dry at room temperature.
7. Proceed to [Counterstaining](#).

Counterstaining:

Apply 15 µl DAPI/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 a lack of signal. Incomplete denaturation of target DNA can result in lack of signal.

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Material provided:

10 test format RtU: 100 µl of probe.
10 test format, 5x concentrated, 20 µl of probe and 100 µl FISH Hybridization Buffer.(FHB)

Probes are labeled with either a green (PlatinumBright 495), red (PlatinumBright 550), or blue (PlatinumBright 415) fluorophore.

Recommendations for Fluorescence Microscopy:

For optimal visualization use a well maintained and regularly calibrated microscope equipped with a 100 W mercury lamp and a x 63 or x 100 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:

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:

- 1% buffered Formaldehyde/1 x PBS/20mM MgCl₂
- PBS
- Xylene
- Formamide
- Ethanol 100%, 85% and 70%
- Fixogum (LK-071A) or rubber cement
- Hot plate (with accurate temperature control up to 80°C)
- Incubator at 37°C + 60°C
- Water bath with accurate temperature at 72°C / 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 (KB-60002):

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

Or

POSEIDON FISH & Digestion kit (KB-60003):

- Pepsin
- 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)
- Counterstain (DAPI/Antifade and Antifade)

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 breathe or allow skin contact. Wear gloves and a lab coat when handling DNA probes and DAPI/Antifade counterstain. Upon disposal, flush with a large volume of water.
3. The use of Sodium Thiocyanate could cause irritation to skin, eyes and respiratory tract and is harmful if swallowed or inhaled. It may affect the heart, blood, thyroid and central nervous system.
4. All materials should be disposed of according to your institution's guidelines for hospital waste disposal.

Patents:

These products or the use of these products is subject to proprietary rights. The probes in these products are produced with the Immunicon Repeat Free™ technology and labeled with the Universal Linkage System (ULS™). The fluorophore used in the PlatinumBright-415 labeling compound is subject to patents, owned or controlled, and manufactured by DYOMICS GmbH. US and International patents pending for Immunicon RF technology. The ULS™ technology and products are covered by US patents 5,580,990; 5,714,327; 5,985,566; 6,133,038; 6,797,818 and several foreign patents owned by KREATECH. KREATECH is a trade name of KREATECH Diagnostics. ULS™ and Poseidon™ are trademarks of KREATECH. Repeat-Free™ is a trademark of Immunicon Corporation. HYBrite™ is a trademark of Vysis, Inc.; Thermobrite™ is a trademark of Statspin.



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