

POSEIDON Repeat-Free™ PROBES

Instructions for use on paraffin embedded tissue sections

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.

Pretreatment:

Recommended when using the KB-60001 POSEIDON Tissue Pretreatment Kit

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

1. Bake 4-5 µm formalin fixed paraffin embedded tissue sections for 2-16 hours at 56°C.
2. De-paraffinize slides by soaking in xylene for 10 minutes two times. Re-hydrate by soaking in 100%, 85% and 70% ethanol for 3 minutes each. Wash with dH₂O for 3 minutes.
3. Pre-treat with 0.2 M HCl for 20 minutes, and then wash in dH₂O for 3 minutes.
4. Place slides in 8% sodium thiocyanate in dH₂O at 80°C for 30 minutes. Rinse in 2 x SSC for 3 minutes.
5. Digest in 0.025% pepsin in 0.01 M HCl at 37°C for 5-45 minutes (Time depending on tissue fixation and tissue type. E.g. most breast cancer tissue need 15 minutes digestion). Wash in dH₂O for 1 minute and in 2 x SSC for 5 minutes.
6. Dehydrate slides by soaking in 70%, 85%, and 100% ethanol for 1 minute each time. Air-dry. Proceed with **Probe preparation:**

Note: Check protein digestion and pre-treatment by applying 15 µl DAPI counterstain and evaluate slides using a fluorescence microscope equipped with a DAPI filter. 15 minutes protein digestion is normally sufficient for a wide range of breast tumors. Remove cover slip and soak tissue in 2 x SSC for 2 minutes and prolong protein digestion by 2-20 minutes if sample is not sufficiently digested. Use a fresh sample and reduce protein digestion time to 10 minutes if the sample is over-digested.

Probe preparation:

POSEIDON 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°C for 5 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 minutes 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 (undiluted at 0.1 µg/ml for tissue) 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.

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:

- 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 and/or 37°
- 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 Tissue

Pretreatment kit (KB-60001):

- Sodium Thiocyanate
- Pepsin
- 0.2 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:

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