BIOSEARCH TECHNOLOGIES

Stellaris® FISH Probes

Protocols and Storage

Catalog No. SMF-2004-1

Product Name

Stellaris FISH Probes, Human PRDM4 with Quasar® 570 Dye

Product Description

Product consists of Quasar 570-labeled oligos mixed and pooled into a final delivered amount of 1 nmol, which yields approximately 80 hybridizations under standard conditions. Designed to detect PRDM4 transcripts in Human specimen using fluorescence *in situ* hybridization (FISH).

Design Criteria

Product was designed against Human PR domain containing 4; PR domain zinc finger protein 4, PRDM4, a.k.a. PFM1, (NCBI gene ID: 11108), and the coding sequence of NM_012406.3 nts 438-2843.

The probe set has not been tested for cross-reactivity to RNA(s) of paralogous and orthologous gene(s) in the same or other species.

Storage Guidelines

Stellaris FISH Probes are shipped dry and may be stored at -20 to +8 °C in this state.

Short-Term Storage & Daily Use

Dissolved probe mix should be subjected to a minimum number of freeze-thaw cycles. For daily and short-term use, the mix can be stored at +2 to +8 °C in the dark for up to a month.

Long-Term Storage

We recommend freezing probes in the dark at -15 to -30 °C for storage lasting longer than a month.

Reconstituting the Dried Probe Stock

The probe stock should be sufficient to provide up to 80 hybridizations. For daily use, aliquots of the probe stock can be stored at +2 to +8 °C in the dark for up to a month.

Redissolve the dried oligonucleotide probe blend in 80 µL of $T_{10}E_1$ buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), to create a probe stock at a total oligo concentration of 12.5 µM. Mix well by pipetting up and down.

1. Reagents and Equipment

1.1. Fixation buffer (50 mL):

Final composition is 3.7% formaldehyde in 1X PBS.

Mix:

5 mL 37% Formaldehyde solution

5 mL 10X Phosphate Buffered Saline (PBS), RNase free

Nuclease-free water to 50 mL final volume

☞ Additionally, for formalin-fixed paraffin embedded (FFPE) tissue sections, proteinase K is required (catalog #P2308-5MG from Sigma-Aldrich or equivalent)

1.2. Hybridization buffer (10 mL):

WARNING! Formamide is a teratogen that is easily absorbed through the skin and should be used in a chemical fume hood.

WARNING! Be sure to let the formamide warm to room temperature before opening the bottle.

Final composition is 100 mg/mL dextran sulfate and 10% formamide in 2X SSC

Mix:

1 g dextran sulfate

1 mL 20X saline-sodium citrate (SSC), nuclease-free

1 mL deionized formamide

Nuclease-free water to 10 mL final volume

1.3. Wash buffer (50 mL):

WARNING! Formamide is a teratogen that is easily absorbed through the skin and should be used in a chemical fume hood.

WARNING! Be sure to let the formamide warm to room temperature before opening the bottle.

Final composition is 10% formamide in 2X SSC

Mix:

5 mL 20X SSC

5 mL deionized formamide

Nuclease-free water to 50 mL final volume

1.4. Nuclear Stain for use after hybridization:

4',6-diamidino-2-phenylindole (DAPI), catalog #D9564 from Sigma or equivalent dissolved at 5 ng/mL in wash buffer

1.5. Anti-fade:

Vectashield® Mounting Medium (Vector Labs, catalog #H1000).

- ☞ Alternately, the GLOX buffer described in the General Protocols and Storage may be used in assays incorporating probes labeled with Quasar 570 and Quasar 670 dyes. The GLOX buffer is incompatible with fluorescein dye.
- ☞ Other commercially available premixed anti-fade solutions such as Cytoseal (RichardAllan or Amazon) or Prolong Gold (Life Technologies) can be used, but have been shown to be less effective than the GLOX buffer, and their use may result in reduced signal.

1.6. Microscope:

- ☞ We **do not recommend using a confocal microscope** with Stellaris FISH probe sets. Confocal microscopes use point illumination to limit the focal plane for imaging. While the technique restricts light that is out of focus, it also diminishes the sensitivity of low-light level imaging.

#1 Round 18 mm coverglass in conjunction with 12-well cell culture plates, catalog #EK-680111 from E&K Scientific or equivalent

Standard fluorescence microscope (e.g., Nikon Eclipse Ti or equivalent)

Strong light source, such as a mercury or metal-halide lamp (xenon or LED are typically not bright enough)

Filter sets appropriate for the fluorophores

Standard cooled CCD camera, ideally optimized for low-light level imaging rather than speed (13 µm pixel size or less is ideal)

60-100x oil-immersion DIC objective or similar

2. Fixation

- ☞ If using a mammalian adherent cell line, proceed to section 2.1: Fixation of Adherent Cell Lines
- ☞ If using frozen tissue sections, proceed to section 2.2: Fixation of frozen tissue sections.
- ☞ If using formalin fixed paraffin embedded (FFPE) tissue sections, proceed to section 2.3: Deparaffinization of FFPE tissue sections

2.1. Fixation of Adherent Cell Lines

a) Grow cells on #1 coverglass in 12-well cell culture plates.
b) Aspirate growth medium and wash with 1 mL of 1X PBS.
c) Add 1 mL of fixation buffer.
d) Incubate at room temperature for 10 minutes.
e) Wash twice with 1 mL of 1X PBS.
f) To permeabilize, immerse cells in 1 mL of 70% ethanol for at least 1 hour at +2 to +8 °C. Cells can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridization.

Proceed to Section 3.1, Hybridization in Adherent Cells.

2.2. Fixation of frozen tissue sections (non-paraffin embedded)

a) Frozen tissue must be sliced at 4 – 10 µm thick using a cryostat.
b) Thaw frozen tissue section to room temperature.
c) Immerse tissue section in fixation buffer for 10 minutes at room temperature.
d) Wash twice with 1X PBS.
e) To permeabilize, immerse the tissue section in 70% ethanol for at least 1 hour at room temperature. Slides can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridization.

Proceed to Section 3.2, Hybridization in Frozen or FFPE Tissue.

2.3. Deparaffinization of formalin-fixed paraffin embedded (FFPE) tissue sections

a) Paraffin embedded tissue must be sliced at 4 – 10 µm thick using a microtome.
b) Immerse tissue section in 100% xylene for 10 minutes; repeat in fresh 100% xylene for an additional 5 minutes.
c) Immerse tissue section in 100% ethanol for 10 minutes; repeat in fresh 100% ethanol for an additional 10 minutes.
d) Immerse tissue section in 95% ethanol for 10 minutes.
e) Immerse tissue section in 70% ethanol for at least 1 hour. Slides can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridization.
f) Immerse tissue section in RNase-free PBS for 2-5 minutes.
g) Decant PBS and immerse tissue sections in pre-warmed (37 °C) proteinase K solution (10 µg/mL in RNase-free PBS).
h) Incubate for 20 minutes at 37 °C.
i) Wash twice with 1X PBS.

Proceed immediately to Section 3.2, Hybridization in Frozen or FFPE Tissue.

3. Hybridization

3.1. Hybridization in Adherent Cells

If refrigerated before using, warm the probe solution to room temperature. Mix well by vortexing and spin down.

Add 0.5 µL of stock probe solution to 50 µL of hybridization buffer, then vortex and centrifuge. This creates a working probe solution with probe concentration 125 nM.

a) Aspirate the 70% ethanol off the cells within the 12-well plates.
b) Add 1 mL of wash buffer and let stand for 2-5 minutes.
c) Assemble humidified chamber, (150 mm tissue culture plate; bottom lined with a water-saturated tissue and a single layer of parafilm above. This will help prevent evaporation of probe solution from coverglass).
d) Dispense 50 µL of the hybridization buffer containing probe onto the parafilm within the humidified chamber.
e) Gently transfer the coverglass onto the 50 µL drop of hybridization buffer containing probe, cells side down.
f) Cover the humidified chamber and seal with parafilm.
g) Incubate in the dark at 37 °C for 4 hours. (For low number of mRNA targets, the incubation can be continued overnight).
h) Gently transfer the coverglass to a fresh 12-well plate containing 1 mL of wash buffer, cells side up.
i) Incubate in the dark at 37 °C for 30 minutes
j) Aspirate the wash buffer, then add 1 mL of DAPI nuclear stain (wash buffer with 5 ng/mL DAPI) to counterstain the nuclei.
k) Incubate in the dark at 37 °C for 30 minutes.
l) Aspirate the DAPI staining buffer, then incubate in 2X SSC.
m) Add a small drop of Vectashield antifade (approximately 25 µL) onto a microscope slide and mount coverglass, cells side down.
n) Seal the coverglass perimeter with clear nailpolish and allow to dry.
o) Gently wipe away any dried salt off the coverglass with water, if necessary.

Proceed immediately to imaging.

3.2. Hybridization in Frozen or FFPE Tissue

If refrigerated before using, warm the probe solution to room temperature. Mix well by vortexing and spin down.

Add 1 µL of stock probe solution to 100 µL of hybridization buffer, then vortex and centrifuge. This creates a working probe solution with probe concentration 125 nM.

a) Immerse the tissue section in wash buffer for 2-5 minutes.
b) Assemble humidified chamber, (150 mm tissue culture plate with a water-saturated tissue. This will help prevent evaporation of probe solution from tissue section).
c) Remove tissue section from wash buffer and carefully wipe away excess buffer surrounding the tissue section.
d) Dispense 100 µL of hybridization buffer containing probe onto the tissue section.
e) Carefully place a clean coverglass over the probe solution covering the tissue section.
f) Place the tissue section in the humidified chamber, cover, and seal with parafilm.
g) Incubate in the dark at 37 °C for 4 hours. (For low number of mRNA targets, the incubation can be continued overnight Immerse tissue section in wash buffer and allow the submerged coverglass to slide off the tissue section. Gentle agitation may be required to remove coverglass.
h) Incubate in the dark at 37 °C for 30 minutes.
i) Decant wash buffer, then add DAPI nuclear stain (wash buffer with 5 ng/mL DAPI) to counterstain the nuclei.
j) Incubate in the dark at 37 °C for 30 minutes.
k) Decant DAPI staining buffer, then immerse tissue section in 2X SSC.
l) Remove tissue section from 2X SSC and carefully wipe away excess 2X SSC surrounding the tissue section.
m) Add a small drop of Vectashield antifade (approximately 25 µL) onto the tissue section and cover with a clean #1 coverglass.
n) Gently squeeze out as much excess Vectashield antifade buffer from underneath the coverglass before sealing the perimeter of the coverglass with clear nail polish

Proceed immediately to imaging.

Technical Support

If you require additional information or technical assistance please feel free to e-mail our Technical Support Group at: techsupport@biosearchtech.com. Our knowledgeable staff is also available for telephone consultation from 8:00 AM to 5:00 PM, Monday through Friday, Pacific Time. Please contact us at:

1.800.GENOME.1 (436.6631) US & Canada only

+1.415.883.8400 telephone

+1.415.883.8488 fax

Sales Support

To place an order or check on the status of an order, please contact our Sales Department by telephone at the numbers shown above, or by e-mail at:

info@biosearchtech.com.

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