BIOSEARCH TECHNOLOGIES

# Stellaris™ FISH Probes

*Protocols and Storage*

## Catalog No. SMF-2014-1

### Product Name

Stellaris™ FISH Probes, Human DHPS with Quasar® 570 Dye

### Product Description

Product consists of 27 Quasar® 570-labeled oligos mixed at equal ratios and pooled into a final delivered amount of 1 nmol, which yields approximately 80 hybridizations under standard conditions. Designed to detect DHPS transcripts in Human specimen using fluorescence *in situ* hybridization (FISH).

### Design Criteria

Product was designed against Human Deoxyhypusine synthase, DHPS, a.k.a. DS; DHS; MIG13, (NCBI gene ID: 1725), and the Common coding sequence, Exons 2-6, and 8-9 of NM_013406.2 nts 325-1210. This is an inclusive probe set designed to also detect the following variants: NM_001206974, NM_001930, NR_038192.

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 vortexing and spin down.

## 1. Reagents and Equipment

### *1.1. Fixation solution (50 mL):*

37% Formaldehyde solution (5 mL), catalog #F8775 from Sigma or equivalent

10x PBS (RNase free) (5 mL), catalog #AM9624 from Ambion or equivalent

Nuclease-free water (to 50 mL final volume), catalog #AM9932 from Ambion 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.

Dextran sulfate (1 g), catalog #D6001 from Sigma or equivalent

20X saline-sodium citrate (SSC) (nuclease-free) (1 mL), catalog #82021-484 from VWR or equivalent

Formamide (deionized) (1 mL for 10% final concentration), catalog #AM9342 from Ambion or equivalent

Nuclease-free water (to 10 mL final volume), catalog #AM9932 from Ambion or equivalent

*>>Continue reading for more information.*

### 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.

20X SSC (5 mL), catalog #82021-484 from VWR or equivalent

Formamide (deionized) (5 mL for 10% final concentration), catalog #AM9342 from Ambion or equivalent

Nuclease-free water (to 50 mL final volume), catalog #AM9932 from Ambion or equivalent

### 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 buffer and enzymes ("GLOX" buffer):

☞ The collection of several images of the same field of view (Z-stacks) requires that the sample is illuminated over an extended period of time. Hence, we recommend the use of reagents to minimize fading of the probes' bright fluorophores. Below is described the protocol for the widely used glucose oxidase catalase system. Other systems may be employed on an experimental basis

10% glucose in nuclease-free water, catalog #158968 from Sigma or equivalent

1 M Tris-HCl, pH 8.0, catalog #AM9855G from Ambion or equivalent

20X SSC, catalog #82021-484 from VWR or equivalent

Nuclease-free water, catalog #AM9932 from Ambion or equivalent

Glucose oxidase (diluted to 3.7 mg/mL in 50 mM sodium acetate, pH ~5), catalog #G0543 from Sigma or equivalent

Catalase, catalog C3155 from Sigma or equivalent

**NOTE:** Mix together 0.85 mL of nuclease-free water and add 100 µL of 20X SSC, 40 µL of 10% glucose and 10 µL of 1 M Tris-HCl. Vortex and then transfer 100 µL of this "GLOX" buffer to another tube, to which one should add 1 µL of the glucose oxidase stock and 1 µL of mildly vortexed catalase suspension. The remainder can be used as an equilibration buffer.

### 1.6. Microscope:

☞ When using an inverted microscope with an adherent cell line:
Lab-Tek chambered coverglass, catalog #43300-772 from VWR or equivalent in conjuction with #1 Square coverslips, catalog # 48366-045 from VWR or equivalent.
**NOTE:** Chambered coverglass is only compatible with an inverted microscope.

☞ When using an upright microscope and/or tissue sections:
#1 Round coverslips, catalog # 48380-046 from VWR or equivalent, in conjunction with cell culture plates from catalog # EK-680111 E&K Scientific, for use on an upright 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.

Standard fluorescence microscope (e.g., Nikon TE2000 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

*>>Continue reading for more information.*

## 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 or in Lab-Tek chambered coverglass (with #1 coverglass on the bottom). The chambered coverglass is ideal for imaging on an inverted microscope.
b) Aspirate growth medium and wash with 1x PBS.
c) Add fixation solution.
d) Incubate at room temperature for 10 minutes.
e) Wash 2x with 1x PBS.
f) Add 70% EtOH and permeabilize at +2 to +8 °C for at least an hour.

*Cells can be stored at +2 to +8 °C in 70% ethanol and hybridized up to a week after permeabilization.*

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

a) Frozen tissue must be sliced into very thin sections of 4 – 10 µm.
b) Thaw frozen tissue section to ambient temperature.
c) Incubate in fixation solution for 10 minutes at ambient temperature.
d) Wash 2x with 1x PBS.
e) To permeabilize, cover the tissue in 70% ethanol for at least 1 hour at ambient temperature. Slides can be stored at +2 to +8 °C in 70% ethanol for up to a week before hybridization.

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

a) Paraffin embedded tissue must be sliced into very thin sections of 4 – 10 µm.
b) Immerse in 100% xylene for 10 minutes; repeat in fresh 100% xylene for additional 5 minutes
c) Immerse in 100% ethanol for 10 minutes; repeat in fresh 100% ethanol for addition 10 minutes
d) Immerse in 95% ethanol for 10 minutes
e) Immerse in 70% ethanol for at least an hour. Slides can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridization.
f) Just before hybridization of the slides, remove slides from the 70% ethanol.
g) Immerse slides in RNase-free PBS for 2-5 minutes
h) Add pre-warmed (37 °C) proteinase K, catalog #P2308-5MG from Sigma-Aldrich or equivalent (10 µg/mL in RNase-free PBS) solution to sections.
i) Incubate for 20 minutes at 37 °C.
j) Equilibrate in wash buffer for 2-5 minutes twice.

*Proceed immediately to Hybridization, step 3.c.*

*>>Continue reading for more information.*

## 3. Hybridization

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 0.125 µM.

a) Aspirate the 70% ethanol off the sample.
b) Add 1 mL of wash buffer and let stand for 2-5 minutes.
c) Aspirate the wash buffer and then add 100 µL of probe in hybridization solution.
d) Place a carefully cleaned coverslip over the sample to prevent drying of the hybridization solution during the incubation.
e) Incubate in a dark humidified chamber at +37 °C for 4 hours. For low number of mRNA targets, the incubation can be continued overnight.
f) Add 1 mL of wash buffer to the sample, remove the coverslip, and incubate again at +37 °C for 30 minutes in the dark.
g) Be sure to remove the coverslip very carefully so as not to disturb the cells underneath.
h) Aspirate the wash buffer, then add 1 mL of DAPI nuclear stain (wash buffer with 5 ng/mL DAPI) to counterstain the nuclei.
i) Incubate at +37 °C for 30 minutes in the dark.
j) Aspirate the DAPI staining buffer and re-suspend in 2X SSC.
k) Aspirate the SSC and add GLOX buffer without enzymes for equilibration; incubate for 1-2 minutes.
l) Aspirate the buffer and re-suspend in the 100 µL of GLOX buffer with enzymes (glucose oxidase and catalase).
m) If using a chambered coverglass:

- ☞ Place a carefully cleaned coverslip over the sample to spread the GLOX buffer over the entire sample and slow evaporation.

If using adherent cells seeded on #1 coverslips:

- ☞ Mount coverslips with GLOX buffer plus enzymes on standard microscope slides, and seal with clear nail polish.

If using frozen or paraffin embedded tissue,

- ☞ Squeeze out as much excess GLOX buffer from underneath the coverslip before sealing with clear nail polish.

## 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

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## 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.