KromaTiD

APPLICATION NOTES

Pinpoint FISH™ DNA Probes – Important Protocol Guidance

Introduction

Pinpoint FISH™ DNA probes are synthetic oligonucleotide-based tools designed to provide the highest resolution, lowest background and lowest limit of signal detection available, with no Cot-1 DNA blocking required. Analysis is easy with highly visible signals using our robust Pinpoint FISH™ DNA probes.

This application note provides guidance on key assay steps in the protocol which deserve special attention. Following the protocol guidance below will reduce artifact cross-hybridization signals and background haze, producing tight, localized signals, saving your lab valuable analysis and troubleshooting time.

Special Notes:

Storing probe solution and hybridization buffer separately.

Do not pre-combine hybridization mix ingredients for long-term storage.

For best performance of our products, KromaTiD provides its probe sets as separate probe and hybridization buffer solution vials, rather than pre-mixing them. Storing probes and hybridization buffer in solution, long-term, risks gradual degradation of your high-value probes through damaging secondary reactions that can take place even at -20°C.

Address your specific experimental question through multiplexing.

Multiplexing KromaTiD Pinpoint FISH™ probes with BAC-based probes

Your project may need data requiring multiple probes working in concert within a cell at once. KromaTiD Pinpoint FISH™ probes can be combined to address your experimental need in a customized way and are even interoperable with BAC-based probes. Read the Multiplexing with Pinpoint FISH probes application note.

Multiplexing KromaTiD Pinpoint FISH™ probes and paints.

All KromaTiD Pinpoint FISH™ can be combined freely. Thanks to their synthetic design process, the sequence of each component oligonucleotide has only one target in the human genome. The Human Whole Chromosome Pinpoint FISH™ Paint Probes require a slightly gentler protocol than the other probe family products. As such, products from the first three categories shown below can be run with the 60°C protocol when combined. Use the 42°C protocol when combining products with a paint probe.

	First Probe Type	Additional Probe Type	Protocol
1	Centromere Pinpoint FISH™ Probes	Probe types 1-3	60°C or 42°C
2	Subtelomere Pinpoint FISH™ Probes	Probe types 1-3	60°C or 42°C
3	Pinpoint FISH™ TP53/CEP17 Kit	Probe types 1-3	60°C or 42°C
4	Human Whole Chromosome Pinpoint FISH™ Paint Probes	Probe types 1-3	42°C

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Hybridization Protocol Guidance: Pinpoint FISH™ DNA Probe Protocols

Use rubber cement to seal coverslips and ensure stable humidity

Some of the risks inherent to using parafilm to seal coverslips are creases, imperfect coverage of the area to be sealed, or accidental shifting of the unmelted parafilm when the slide is placed onto the denaturation hot plate. A safer choice is rubber cement. When applying, ensure coverage overhangs the edges of the coverslip but avoid spillage over the slide edge. Rubber cement that gets under the slide will prevent full contact between the slide and the hot plate. Routinely check the hot plate for residue from past runs to ensure this is not happening in your lab.

Controlling ambient humidity during the denaturation and reannealing steps reduces analysis time and prevents costly repeat-hybridization runs. Much better assay results will be obtained when slides incubate in a humidity chamber rather than on a standard denaturation device. Popular denaturation hot plates have a humidity card/strip alongside the slides but this is not enough to guarantee sufficient humidity control.

Preventing ink and slide label adhesive contamination.

It is common practice to prop slides up vertically, label-end up, after pulling them out of a solution. What can often result is contaminant chemicals like slide adhesive and marker ink forming a visible streak down the surface of your precious sample. To protect against this needless risk, prop slides up vertically, with the label-end down, to dry or gently dab the label with a dry paper towel before they are placed in a label-end up position.

Removing bubbles under a coverslip

Air bubbles under a coverslip create spaces that are lost to the total area available for analysis and it is tempting to press on the coverslip to force them out. Doing this may inadvertently grind cells between the two layers of glass if the coverslip slides suddenly. If there are likely to be enough cells available despite the bubbles, doing nothing may be better for the overall value of the work already invested.

Post-Hybridization Wash Protocol Guidance

Water bath temperature

Pinpoint FISH™ probes are designed as target-specific oligonucleotides that will anneal best when they are washed post-hybridization according to precise protocol conditions, which may vary from those of another product.

Centromere and Subtelomere Probes
Use post-hybridization wash (0.4X SSC/ 0.05% Tween-20) at **60°C**. See 60°C protocol.

Human Whole Chromosome Paint Probes
Use post-hybridization wash (2X SSC) at **42°C**. See 42°C protocol.

Microscope Filter Guidance for Slide Imaging: All Pinpoint FISH™ DNA Probes

Your lab's time and resources are very valuable. To set you up for success, KromaTiD provides exact wavelength specifications on our protocols for each color label offered. Confirming that these align with your microscope filters will help your KromaTiD products serve you best.

Fluorophore Information

Fluorophore	Excitation/Emission (nm)
Atto425 / Aqua	436 / 485
6-FAM / Spectrum Green	490 / 525
Atto550 / Spectrum Orange	555 / 576
TexRed	595 / 620
Atto643 / 647 / Cy5	643 / 669

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