

APPLICATION NOTES

Pinpoint FISH™ DNA Probes – Water Bath Guidance

Post-Hybridization Wash Guidance: Pinpoint FISH™ DNA Probes

KromaTiD's Pinpoint FISH™ DNA Probes are high-precision tools that offer a level of resolution unrivaled by mainstream FISH probes on the market. The post-hybridization wash steps of the Pinpoint FISH™ protocols are critical to achieving this high level of performance. This guidance document is meant to offer best practice tips when using a water bath during post-hybridization washing.

Which Type of Water Bath to Use

During the post-hybridization wash, the variable that the water bath can influence most is the uniformity of heat distribution. The three types of water baths most often found in laboratories (shaking, non-circulating, and circulating water baths) differ in how well they accomplish the task of heating samples.

Shaking water baths are optimized to facilitate gas exchange between the air and microbes in a liquid culture. A shaking water bath can be used for a post-hybridization wash but switching off the shaking is strongly advised. Otherwise, the greater interaction between wash solutions and the air may create a large temperature gradient in the liquid, lowering the temperature the nearer to the surface the fluid is.

Non-circulating water baths are the most common type of bath found in laboratories. These rely on convection to move heat from the heating element(s) beneath the water basin upwards throughout the water. If a non-circulating water bath has a stirring function, such as a spinning magnet to agitate a stir bar placed in the solution container then this is an excellent way to distribute heat evenly throughout the liquid.

Circulating water baths are less common but are optimized for even heat distribution. Physically moving the water around within the water basin, a circulating water bath disturbs any pockets of colder or warmer water.

Ensuring Accurate Temperature Measurement

Most water baths come with a digital temperature display, and/or an attachment that can hold a physical thermometer. It is not advised to rely on these alone. A thermometer or electronic temperature probe should be used. It should be certified by the National Institute of Standards and Technology (NIST), or other appropriate authority on instrument accuracy.

The glass thermometer or the probe-end of the electronic thermometer should be mostly submerged in the wash solution when measuring temperature, rather than just in the basin water, especially if waiting for the water bath to get up to the target temperature. The basin water will usually reach the target *before* the wash solution does. Care must also be taken to avoid physically touching anything other than wash solution with the measuring instrument. Contact with solid objects, especially metal may transfer heat faster, giving a different reading than the solution alone would. Lastly, filling the basin enough for the wash solution container to be at least 75% submerged will help keep the wash solution uniformly heated.

application notes

Water Bath Temperatures for Pinpoint FISH™ DNA Probes

Regardless of the probe type, the post-hybridization wash portion of all KromaTiD Pinpoint FISH™ assays involves at least one heated wash step. At a glance, these are the post-hybridization wash steps for each probe type that require heating in a water bath.

Probe Product Category	Heated Post-Hybridization Wash Step(s)	Links
Centromere Enumeration	1 x 0.4xSSC/0.05% Tween at 60°C	60°C Protocol
Subtelomere	1 x 0.4xSSC/0.05% Tween at 60°C	60°C Protocol
tp53/CEP17 Kit	1 x 0.4xSSC/0.05% Tween at 60°C	60°C Protocol
Chromosome Paint	5 x 2xSSC at 42°C	42°C Protocol
Multiplex	Use 42°C protocol if multiplexing with a paint probe	varies

Multiplexing KromaTiD Pinpoint FISH™ Probes

All KromaTiD Pinpoint FISH™ probes can be combined into the same hybridization mix. KromaTiD customers have the ability to custom-design their own probe tool combinations to generate the specific data sets needed to answer their unique experimental questions. As described in the [Multiplexing With KromaTiD Pinpoint FISH™ Probes](#) application note, probes need only be combined, dehydrated, and then rehydrated to the appropriate volume. After that, the applicable protocol can be followed as written. All KromaTiD Pinpoint FISH™ probes will work optimally when following the [42°C protocol](#). If the probe combination does not include whole chromosome paints, the briefer [60°C protocol](#) may be employed.