

FISH Troubleshooting FAQ's

Problem	Possible Reason	What to do
Distorted chromosome morphology	Slides dried too quickly? Assess slides with phase contrast microscope View FISH Slide Preparation Protocol	Increase humidity when dropping slides – refer to slide making procedure
		Increase time after dropping slide before placing on slide warmer.
		Ensure slide warmer is ~45°C. Monitor temperature with surface thermometer.
		Refix cell suspension in freshly prepared 3:1 methanol: glacial acetic acid fixative.
	Slides aged/stored improperly	Age slides at least 24 hrs at room temperature prior to FISH.
		Do not bake slides.
		If FISH needs to be performed the same day as slide preparation, incubate slide at least 1 hr in a coplin jar containing 2 x SSC 37°C then dehydrate slide for 1 minute in 70%, 85% and 100% ethanol prior to denaturation step.
	Overdenaturation	Prepare denaturation solution made according to package insert
		Ensure temperature of denaturation solution is 73±1°C.
		Decrease temperature of denaturation solution to 72°C.
Decrease time in denaturation solution to 4 minutes.		

High Background	Glass slides not clean	Soak slides in 70% ethanol 5 min then wipe vigorously 2-3 times with a tissue.
	Poor specimen quality with cellular debris	Wash cell pellet with fresh fixative 2-3 times then repeat slide making. Ensure that cell suspension is not too thick.
	Cytoplasm around chromosomes	Slides dried too quickly. (View FISH Slide Preparation Protocol)
	Wash Solutions	Ensure WS1 & WS2 are prepared & stored properly.
		Check pH (7.0) and temperature ($73 \pm 1^\circ\text{C}$) of wash solutions are correct. Place thermometer directly in WS1.
		Ensure wash solutions are not expired or over used (discard after 10 slides).
		Ensure only 4 slides are washed at once to maintain proper temperature of WS1.
		Ensure time in wash solutions are appropriate. (Review Hybridization Protocol)
		Increase time in WS1 to 3 minutes.
	Broad Band Pass microscope filters	Use filters with narrow band widths specific to fluorochromes used. Contact info@ Empire Genomics
Latex Gloves	Contamination with latex particles appear as a fine yellow fluorescent precipitate over the slide. Use powder free gloves.	
Too much probe	Follow set up protocol (Review Hybridization Protocol)	
Inadequate hybridization conditions	Use tightly sealed chamber with appropriate humidity control	

Weak or no signal	Incorrect specimen preparation	Refer to link below for evaluating specimen processing, slide making, slide aging (View FISH Slide Preparation Protocol)
	Slide not adequately denatured	Ensure denaturation buffer is prepared correctly with proper pH7.0.
		Ensure temperature of denaturation buffer is (73±1°C). Insert thermometer directly into solution. Increase temperature of denaturation buffer to 74°C.
		Ensure time in denaturation buffer is correct (5 min). Increase time in solution by 2-4 minutes.
	Probe not adequately prepared	Completely thaw probe and hyb buffer (15 min. RT). Add hybridization buffer to dehydrated probe. Pipet vigorously to mix & incubate for 15 min at 37°C. Vortex & centrifuge briefly.
	Probe not adequately denatured	Ensure temperature of water bath used to denature probe is (73±1°C).
		Ensure time in water bath is 5 minutes.
	Probe not added	Add probe to slide immediately after denaturation. Ensure air bubbles are removed.
	Incorrect wash conditions	Ensure wash solutions are fresh and prepared properly
		Ensure temperatures (73±1°C) and times (2 min WS1/1 min WS2) are correct in wash solutions. (Review Hybridization Protocol)
Ensure coverslip is removed before placing slide in wash solution.		
Probe exposed to light or stored incorrectly	Perform all FISH procedures in a dimly lit room. Store probes at -	

		20°C. Avoid excessive freeze/thaw cycles.
	Microscope specifications inadequate	Ensure UV light source is adequate for viewing FISH signals. Contact your microscope manufacturer.
		Ensure UV light source is centered.
		Ensure proper filters are installed for fluorochromes used. Contact microscope manufacturer.
		Ensure filters are not damaged. Contact microscope manufacturer.
		Ensure wash solutions were prepared properly.
Low signal specificity	Wash solution stringency too low	Note: the lower the [conc] of SSC, the higher the [conc] of formamide and NP-40, the more stringent the wash.
		Ensure probe mixture was made properly.
	Probes diluted improperly	Wash only up to 4 slides at once to maintain proper temperature of hot wash solution. Ensure temperature of WS1 is correct prior to addition of slides by inserting thermometer directly into coplin jar.
	Wash solution temperature is too low	Remove coverslip. Dehydrate slide through ascending alcohols 1 minute each (70%, 85%, 100%). Air dry and reapply counterstain.

Counterstain too weak or bright	Slides not dried completely prior to application of counterstain	If too bright, dilute DAPI with antifade solution before applying.
	Wrong concentration of counterstain	If too weak purchase a more concentrated DAPI solution. Ensure DAPI stock is not exposed to light for extended periods. Ensure DAPI is not past expiration date.