Vysis CEP Chromosome Enumeration DNA Probe Troubleshooting Guide

The chromosome morphology observed in hybridization where codenaturation is used may differ from a specimen that is denatured and dehydrated before the probe is applied. Often, this has little effect on the analysis on interphase cells. Additionally, probe targets that are over-denatured may exhibit “speckling,” in which the hybridized probe appears as brightly stained spots with smaller spots radiating from the central spots. Speckling is more likely to occur when using higher intensity probes, such as a SpectrumOrange probe. Some speckling should not interfere with chromosome enumeration.

Problem	Possible Solution
Cross hybridization	Repeat on a new specimen using one of the following: Increase the temperature of 0.4X SSC/0.3% NP-40 by 2°C. As needed, continue to increase the temperature until the cross hybridization is minimal. Decrease the denaturation temperature by 2°C. Make sure incubation is at 42°C. Ensure CEP Hybirdization Buffer was used.
Probe appears dim	Repeat on a new specimen using one of the following: Increase the hybridization time. Increase the denaturation temperature. As needed, continue to increase the temperature until the signal becomes acceptable. Increase the denaturation time. Pretreat this specimen. Call Technical Service for assistance. Wash the slide using 0.4X SSC/0.3% NP-40 at 70-73°C Ensure filters are correct and not damaged.
Diffuse signal (speckling)	Repeat on a new specimen using one of the following: Decrease the denaturation temperature 2°C. Decrease the denaturation time. **NOTE:** As needed, reduce the denaturation temperature or time until metaphase morphology has improved and signal intensity is stil acceptable. Wash the slide using 0.4X SSC/0.3% NP-40 at 73–76°C.
Poor metaphase morphology	Repeat on a new specimen using one of the following: Decrease the denaturation temperature 2°C. Decrease the denaturation time. **NOTE:** As needed, reduce the denaturation temperature or time until metaphase morphology has improved and signal intensity becomes acceptable. Age the slides: 1. Prepare a solution of 2X SSC. 2. Immerse the slide in 2X SSC for 2 minutes at 73°C. 3. Immerse the slide several times in purified water. 4. Dehydrate slides through a series of 1 minute EtOH rinses (70%, 85%, 100%). 5. Air dry the slides and continue with hybridization.

When performing FISH on interphase cells, samples containing DNA that is damaged, degraded or destroyed by extreme conditions or treatments such as acid, strong alkali, and heat will not produce FISH results. Icteric, hemolyzed or lipemic specimens may prevent proper culture for standard cytogenetic analysis, but will still provide an acceptable FISH result. Clotted or frozen specimens may lead to poor FISH results, but unclotting or thawing the specimens can achieve successful FISH results.

When viewing FISH results, ensure that your microscope is properly aligned and functioning optimally.

The following table lists some examples of variable results that you may encounter using the CEP probes. Probable causes and suggestions to improve performance are included.

Problem	Probable Cause	Possible Solution
Distorted chromosome morphology	Specimen slides dried too quickly during preparation.	Increase temperature of water bath (increases humidity) used when dropping slides. Decrease the temperature of the slide warmer during sample preparation. Increase drying time to at least overnight at ambient temperature, and then age slides at least 24 hours at ambient temperature. Do not bake slides at high temperature.
	Specimen over-denatured.	Ensure the denaturation solution was made correctly. Ensure temperature of denaturation solution is 73±1°C prior to immersing the slide; decrease the temperature to 72°C. Decrease the time the slide is immersed in the denaturation solution by 1–3 minutes.
	Specimen slides too fresh prior to denaturation.	Age slides at least 24 hours at ambient temperature or artificially age the specimen in 2X SSC at 73°C for 2 minutes.
	Specimen slides not thoroughly dry prior to immersion in denaturation solution.	Warm specimen slides to 45–50°C prior to denaturation or dehydrate slides through a series of 1 minute EtOH rinses (70%, 85%, 100%).
High slide background	Glass slides not sufficiently cleaned prior to sample preparation	Immerse glass slides in EtOH and wipe dry using lint-free paper prior to dropping slides.
	Cellular debris in sample preparation.	Wash cell pellet with fresh fixative three times and repeat the slide dropping procedure.
	Metaphase spreads were aged by baking or contain cytoplasm.	Increase time the slide is immersed in the denaturation solution up to 10 minutes.

Problem	Probable Cause	Possible Solution
High slide background	Slide inadequately washed following hybridization.	Ensure the wash solutions were made correctly. Ensure pH and temperature of wash solutions are correct. Remove coverslip. Repeat the wash procedure. Increase the time that the slide is immersed in the 73˚C 0.4X SSC/0.3% NP-40 wash solution up to 4 minutes.
	Wash solutions used too long or stored improperly.	Discard all wash solutions after 1 day.
	Viewed hybridization using long bandpass filters.	Switch to filters with narrow bandwidths or to multi-bandpass filters to reduce background light.
Weak or no signal	Specimen slide not adequately denatured.	Ensure temperature of denaturation solution in the Coplin jar is 73±1˚C prior to immersing the slide. Increase temperature of denaturation solution to 74˚C. Increase the time the slide is immersed in the denaturation solution by 2-4 minutes.
	Specimen slides improperly aged after dropping specimen.	Age for 24 hours at ambient temperature or artificially age the specimen in 2X SSC at 73˚C for 2 minutes, before performing FISH on them.
	Specimen slides not thoroughly dry prior to immersion in denaturation solution.	Warm specimen slides to 45-50˚C prior to denaturation or dehydrate slides through a series of 1 minute EtOH rinses (70%, 85%, 100%).
	Specimen was GTG-banded.	Use of trypsin-Giemsa banded specimens for FISH may require adjustments in banding and/or hybridization protocols. Prepare fresh specimen slides.
	Probe not added.	Prepare a new probe mixture. Allow the probe to thaw completely. Vortex or pipet reagents to mix; centrifuge briefly. Pipet probe slowly.
	Probe and hybridization buffer were not mixed well prior to use.	Vortex or pipet reagents to mix; centrifuge briefly.
	Probes improperly diluted for hybridization.	Use the volumes stated in the procedure to maintain the ratio of the probe mix (7 µL hybridization buffer: 1 µL probe: 2 µL purified H_2O). Ensure the pipet is calibrated. Allow hybridization buffer to thaw completely and to reach ambient temperature prior to use; pipet slowly.
	Probe not adequately denatured.	Ensure temperature of the water bath used to denature the probe mix is 73±1˚C. Denature the probe mixture for 5 minutes.
	Probe not applied to the target sample immediately after the probe was denatured.	Plan so the probe is applied immediately after the slides are removed from the 100% EtOH solution. Ensure the EtOH has evaporated before applying probe. Remove tube containing probe mix from 73±1˚C water bath and immediately place the tube on a 45–50˚C slide warmer. Keep the tube on the slide warmer while pipetting the probe onto the slide. Process only as many slides as you can and still maintain the correct temperatures and times according to the procedure.
	Probe mix dried too much on the specimen slide.	Immediately place the coverslip over the target area after applying probe mix. When washing slides after the hybridization, remove the coverslip from one slide at a time and immediately immerse the slide into the wash solution before removing the coverslip from the next slide.
	Air bubbles were trapped under the coverslip during hybridization.	Apply coverslip by first touching the surface of the probe. Place the slide with the coverslip down on a blotter and very gently press out visible bubbles.
	Hybridization conditions inappropriate.	Ensure that the correct time and temperatures for the hybridization were followed. Ensure that the temperature of the incubator is 42˚C. Seal the coverslip well with rubber cement, leaving no gaps. Increase the hybridization time.
	Wash conditions or solutions incorrect.	Ensure that the wash solutions were made correctly. Ensure the temperatures of the wash solutions are at the stated temperatures. Ensure that the thermometers and pH meters used are calibrated properly. Remove coverslips before immersing slides in wash solution.

Problem	Probable Cause	Possible Solution
Weak or no signal	Probes or specimen slides stored improperly.	Store undiluted probe at –20°C in the dark. Store non-hybridized slides desiccated at -20°C for an extended period or at ambient temperature for short periods. Store hybridized slides at -20°C in the dark for up to 6 months.
	Wrong counterstain used or counterstain is too bright.	Remove coverslip. Immerse slides for 5 minutes in 2X SSC/0.1% NP-40 at ambient temperature; dehydrate slide through a series of 1 minute EtOH rinses (70%, 85%, 100%). Air dry and reapply a different counterstain.
	Viewed hybridization using inappropriate filter set.	Multi-bandpass filter sets provide less light than single bandpass filter sets, so probe signals may appear fainter when viewed through the multi-bandpass sets. Use correct filter for viewing the probe fluorophore.
	Microscope configuration or objectives not adequate for viewing FISH results, or microscope filters are damaged.	Contact your microscope manufacturer.
Low signal specificity	Probes diluted inappropriately; often too much probe.	Ensure the probe mixture was made correctly.
	Inappropriate hybridization conditions.	Ensure temperature of incubator is 42°C. Ensure that the CEP Hybridization buffer was added to the probe and in the proper amount.
	Wash temperature too low.	Maintain the wash temperature of the wash solutions by placing no more than four slides in one Coplin jar at a time and ensuring that the temperature of the wash solution is correct before washing another set of slides.
	Wash solution stringency too low.	Ensure the wash solutions were made correctly. **NOTE:** The lower the concentration of salt (SSC), the higher the concentration of NP-40, the more stringent the wash.
Bright or weak counterstain	Counterstain appears weak: specimen slides not dehydrated prior to applying counterstain or oil droplets in counterstain. Wrong concentration of counterstain. **NOTE:** DAPI I counterstain is eight times more concentrated than DAPI II counterstain.	Remove coverslip. Immerse slides for 5 minutes in 2X SSC/0.1% NP-40 at ambient temperature; dehydrate slide through a series of 1 minute EtOH rinses (70%, 85%, 100%). Air dry and reapply counterstain. Ensure the correct counterstain is being used.
	Counterstain too old or exposed to light for extended periods.	Store counterstain at -20°C in the dark. Ensure the counterstain is not used past the expiration date. DAPI turns dark as it degrades.

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