



Fluorescent BAC DNA FISH Probes

Instruction Guide

Empire Genomics, LLC

701 Ellicott Street Suite 203
Buffalo, NY 14203

Technical Support

Phone: 1-800-715-5880
Fax: 716-849-6890
Email: info@empiregenomics.com
<http://www.empiregenomics.com.com>

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BAC DNA Fluorescent Probes

Hybridization Protocol for Labeled FISH Probes

A. Probe Preparation

Fluorescently-labeled BAC DNA probes (~ 1 µg) and Cot-1 (3 µg) are provided in 10 µL of TE (10 mM Tris, pH 8.0, 1 mM EDTA). The amount provided is sufficient for 5 probe hybridizations with metaphase spreads. To prepare probe, add 8 µL of hybridization buffer to 2 µL of probe in a separate tube for a total volume of 10 µL.

Addition of CEN probe is optional. For addition of CEN probe, reduce the amount of hybridization buffer to 7 µL and add 1 µL of labeled CEN probe (12.5-50 ng) to the 2 µL of probe for a total volume of 10 µL.

B. Recommendations for Hybridization of BAC DNA Probes

1. Denaturation of Probe

- a. Denature the probe by heating the mix for 5 minutes at 73°C in a water bath or a heating block with heated lid.
- b. Place the tube on ice for ~ 2 min. Centrifuge briefly.
- c. Pre-warm the denatured probe mix at 37°C for ≥15 minutes before hybridization to the metaphase spreads.

2. Hybridization of probes to metaphase chromosomes

- a. Bring slides containing metaphase spreads to room temperature.
- b. Pre-warm the denaturation buffer (70% formamide, 2x SSC, pH 7.0-8.0) at 75°C for 30 min. Immerse the slides into the hybridization solution for 5 minutes.
- c. Place the slides in 70% ethanol for 1 minute, followed by 85% ethanol for 1 minute and 100% ethanol for 1 minute for dehydration.
- d. Touch the bottom end of the slides to tissue paper sheet to dry the slide. Wipe the underside of the slide with a paper towel.
- e. Place the slide on a slide warmer heated to ~45°C until remnants of ethanol to evaporate.
- f. Apply 10 µL of the denatured probe (from step 1h) on to the slide.
- g. Apply a clean 22 x 22 mm coverslip and place the slide in a sealed and humidified slide chamber. Incubate at 37°C for 16 hours. Use a solution of 50% formamide, 2X SSC as a humidity control.

3. Post Hybridization Wash

- a. Pre-warm Wash Solution 1 (0.4X SSC, 0.3% NP40) at 73°C for 30 minutes or until cloudy. Add Wash Solution 2 (2X SSC/0.1% NP40) to a separate container.
- b. Disassemble the slide chamber, remove the coverslip and place the slide in Wash Solution 1, taking care that the slide does not dry out. Agitate the slide for 1-3 seconds and let stand for 2 minutes at 73°C.
- c. Transfer the slide to container with Wash Solution 2. Agitate for 1-3 seconds and let stand at room temperature for 1 minute.
- d. Dry the slides in the dark.

4. Counterstaining the Chromosomes

- a. Apply 10 µL of mounting medium containing DAPI (125 ng/ml) and antifade reagent.

- b. Coverslip each hybridization location.
- c. Visualize under microscope using appropriate fluorescent filter sets.

C. Troubleshooting Guide

Problem	Potential Cause	Suggestion
High Background	Too much probe in hybridization	Use less probe
	Insufficient Cot-1	Increase amount of Cot-1
	Inadequate hybridization conditions	Use tightly sealed chamber with appropriate humidity control
	Insufficient stringency in washing	Increase stringency of wash solutions and/or increase wash temperature
Low Signal	Not enough probe in hybridization	Increase amount of probe in hybridization
	Stringency of hybridization buffer or wash solutions too high	Decrease stringency of hybridization buffer or wash solutions, ensure temperatures are correct
	Inadequate denaturation of slide or probe	Increase denaturation temperature to 74°C
	Inappropriate filter set on microscope	Multiple bandpass filter sets provide less light than single bandpass. Use correct filters for fluorophores.

Notes

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Quick Reference - Hybridization Protocol

Slides

Probes

