

ChromoTrax

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PROTOCOL FOR DIRECT LABEL PROBES

Product Description

ChromoTrax FISH probes are generated by chromosome microdissection or from BAC clones. The DNA fragments have been mixed with the hybridization buffer. After hybridization with denatured samples, the probes can be used to detect chromosome numeric changes and chromosomal rearrangements.

Three direct labels, tetramethylrhodamine, Sulforhodamine 101 (Texas red), and Fluorescein, are available.

- Tetramethylrhodamine: orange red fluor; excitation peak 546nm; emission peak 576nm
- Sulforhodamine 101: red fluor; excitation peak 595nm; emission peak 615nm
- Fluorescein: green fluor; excitation peak 504nm; emission peak 532nm

Warning & Precaution

Fluorophores may be photo-bleached when exposed to excessive light. Handling reagents and slides containing fluorophores in the dark may help to reduce this effect.

Storage

ChromoTrax FISH probes should be stored at -20°C and protected from light exposure. See the expiration date on the product label.

Quality Control

All ChromoTrax FISH probes are tested by hybridizing to normal human metaphase spreads prepared from lymphocytes.

Reagents

20 X SSC, pH7.0

Dissolve 175.3g Sodium Chloride and 88.2g Sodium Citrate in 800ml dH₂O and adjust the pH to 7.0 with 10N NaOH. Adjust the volume to 1L with dH₂O. Sterilize by autoclaving.

70%, 85% ethanol (stored at room temperature)

Prepare by adding dH₂O to 700ml or 850ml absolute ethanol to a final volume of 1L.

Denaturing solution: 70% (v/v) formamide, 2 X SSC (pH7.0), 0.1mM EDTA, pH7.0

Mix 35ml formamide, 5ml 20 X SSC (pH7.0), 10µl 0.5M EDTA, pH7.0, and 10ml dH₂O to make 50ml solution. Verify that the pH is 7.0-7.5 by measuring the pH at ambient temperature. Between uses, store at 4°C. Discard after 1 month.

Wash Solution (WS): 2 X SSC / 0.1% Tween 20

Mix 50ml 20 X SSC and 500µl Tween 20, adjust the volume to 500ml with dH₂O.

Protocol

Specimen Preparation

1. Prepare metaphase spreads. Age at 37°C for 1-7 days.
2. Equilibrate denaturing solution in a 75±2°C water bath for approximately 30 minutes.
3. Immerse slides in denaturing solution for 2 minutes.
NOTE: Denaturing time should be increase 1-3 minutes if the age of slide is more than one week.
4. Dehydrate slides by incubating slides in 75%, 85% and 100% ethanol for 1 minute in each ethanol concentration.
5. Air dry slides.
NOTE: Slides should be dried thoroughly to get best FISH result.

Probe Denaturing

1. Denature 10µl FISH probes (3µl for 5-color PGD probes) in a 75±2°C for 5 minutes.
2. Keep the probes at 37°C for 2 minutes.

Hybridizing Probes to the Specimen

1. Apply the denatured probe to the slide.
2. Immediately apply a coverslip and seal with rubber cement. Keep the slide in a moist chamber at 37°C overnight for hybridization.

Slide Washing

1. Prepare two tanks of Wash Solution (WS).
2. Place one of the wash tanks containing WS in a 72°C water bath for at least 30 minutes prior to use.
3. Remove rubber cement and cover slips.
NOTE: To avoid drying, slides can be kept temporarily in WS at ambient temperature.
4. Wash the slides with WS at 72°C for 2 minutes.
5. Wash the slides with WS at ambient temperature for 2 minute.
6. Dehydrate slides by incubating slides in 75%, 85% and 100% ethanol for approximately 1 minute in each ethanol concentration.
7. Air dry the slides in darkness.

Visualization

1. Apply 20µl of DAPI counterstain and a coverslip to hybridization location.
NOTE: for 5-color PGD probes, antifade solution should be applied instead of DAPI counterstain.
2. Store in darkness for 20 minutes; afterwards, examine the slides under a fluorescence microscope with proper filter set.

Troubleshooting

Problem	Possible Cause	Possible Solution
Chromosome morphology distorted	Specimen overdenatured	* Decrease denaturing temperature to 72°C * Decrease the time of slide denaturing
High slide background	Slide not clean prior to use	* Immerse slide to 100% ethanol prior to use
	Too much cell debris on sample	* Wash cell pellet with fresh fixative several times before making metaphase spreads
	Inadequate wash after hybridization	* Remove coverslip, and wash again * Ensure the pH and temperature of wash solution * Use fresh wash solution
Signal weak or invisible	Aging mercury lamp (the usage of mercury lamp should not exceed 200 hours)	* Change the UV lamp
	Exhausted filter	* Change the filter
	Incorrect filter used (i.e. Texas filter should not be used for Rhodamine, and vice versa)	* Use the correct filter or choose the proper fluorophore for the filter.
	Inadequate denaturing of slides	* Increase denaturing temperature * Increase denaturing time * Use fresh denature solution
	Probes not added	* Add probes to sample slides
	Inadequate denaturing of probes	* Ensure probe denaturing temperature is 75°C * Increase probe denaturing time by 2 minutes
	Probes dried	* Ensure coverslip is sealed by rubber cement * Ensure moist chamber has ample water
	Air bubbles during hybridization	* Ensure coverslips are cleaned before use * Gently place coverslip gently during hybridization
	Probes expired	* Check the expiration date on Product Information label
	Probes improperly stored	* Store probes at -20°C