

Fluorescent in situ hybridization (FISH) protocol

ULS™-dGreen and ULS™-Rhodamine labeled probes for translocation, locus specific and chromosome copy number detection

ULS™-dGreen and/or ULS™-Rhodamine labeled ready-to-use probes are tested for co-denaturation of probe and target on a temperature controlled hot plate, thus avoiding use of formamide during pre and post hybridization steps. Probe performance was also tested under denaturation conditions using 70% formamide/2X SSC. It is important to view all the target slides under phase contrast for sparse cells and visible cytoplasm before proceeding to FISH hybridization.

Protocol I & II

Gloves
Ethanol
20X SSC
10% SDS
Coplin Jar
Water bath
DAPI/antifade
Micro centrifuge
Deionized formamide*
0.5ml microfuge tube
Autoclaved distilled water
Cover slip 22x22 & 24x50
Temp. controlled hot plate

Additional items for Protocol I

Tween 20
Hydrochloric acid
37% Formaldehyde
Pepsin
Phosphate Buffered Saline

In addition to the items required for protocol I the above items are required for protocol II

*required for formamide denaturation only

PROTOCOL I:

For cells with visible cytoplasm surrounding inter-phase and metaphase

Slide Preparation

1. Incubate slide in 0.01M HCl with 0.005% pepsin at 37°C for 10 min.
2. Wash slide 2 x 1 min in PBS at RT.
3. Incubate slide for 10 min in 1% formaldehyde in PBS at RT.
4. Wash slide for 2 x 1 min in PBS at RT.
5. Dehydrate slides in 70%, 95%, 100% Ethanol at RT for 1 min each.
6. Air dry.

Probe Denaturation/ Hybridization

1. Aliquot 10 µl of probe for each target into 0.5 ml microfuge tube.
2. Incubate at 96°C for 5 min in water bath.
3. Shortly spin the tube in a micro centrifuge.
4. Apply 10 µl of the probe mix to each target and cover with a cover-slip (22x22mm).

Procedure Note: Do not heat probes for more than 5 minutes.

5. Denature slide and probe for 2 min at 80°C on a temperature controlled hot plate.
6. Incubate for 12-18 hours in a humidified environment at 37°C.

Post Hybridization Washing

1. Remove cover-slip by soaking in 2X SSC/0.1% Tween-20 at 37°C.
2. Wash slide 4 x 5 min in 0.5X SSC/0.1% SDS at 60-65°C.
3. Briefly rinse slides in distilled water.
4. Air dry slides out of direct light.
5. Apply 20 µl DAPI/anti fade solutions to the target and cover with a cover slip. (24x50mm).

Storage of slides

Store hybridized slides at -20°C in dark.

Storage of Probe

Store probe at 4 °C

The DNA for the probes in this product was manufactured by Cancer Genetics, Inc, Cambridge, MA, USA. The probes in this product are labeled with the Universal Linkage System (ULS®) and manufactured by KREATECH Biotechnology BV, Amsterdam, The Netherlands. ULS and KREATECH are registered trade marks of KREATECH Biotechnology BV, Amsterdam, The Netherlands.

2004 Rev. 3.07

PROTOCOL II:

For fresh slides from cultured cells and from direct harvest.

This protocol is recommended for cultured cells fixed in Methanol:Acetic Acid (3:1) and for cells prepared by direct harvest. For samples requiring same day hybridization or with-in 24 hours, pre-treatment in 2X SSC at 37°C for 2-3 hours is required for better probe performance and consistent results.

Slide Preparation

Procedure Note: If slides are pretreated in 2X SSC rinse briefly in distilled water then proceed to dehydration

Method A. Probe Denaturation and Hybridization (Hot plate method)

Slide denaturation

1. Dehydrate slide in 70%, 80% and 100% Ethanol at RT for 1 min each.
2. Air dry.
3. Aliquot 10 µl of probe for each slide into 0.5 ml microfuge tube.
4. Incubate the tube at 96°C for 5 min in water bath.

Procedure Note: Do not heat probes for more than 5 min.

5. Shortly spin the tube in a micro centrifuge.
6. Apply probe mix to each slide and cover with a cover-slip (22x22mm).

Procedure Note:

Preset the hot plate to the required temperature.

7. Denature slide and probe for 2 min at 80°C on a temperature controlled hot plate.
8. Incubate for 12-18 hours in a humidified environment at 37°C.

Method B. Probe Denaturation and Hybridization (Formamide method)

Slide denaturation

1. Dehydrate slide in 70%, 80% and 100% Ethanol at RT for 1 min each.
2. Air dry for 5 min.
3. Denature slides in denaturing solution for 2-3 min at 75°C.
4. Dehydrate in cold alcohol series (70%, 80% and 100%)
5. Air dry.

Probe denaturation

1. Aliquot 10 µl of probe for each slide into 0.5 ml microfuge tube.
2. Incubate the tube at 75°C for 7 min in water bath.
3. Incubate at 37°C for 10 min.
4. Shortly spin the tube in a micro centrifuge.
5. Apply probe mix to each slide and cover with a cover-slip (22x22mm).
7. Incubate for 12-18 hours in a humidified environment at 37°C.

Post Hybridization Wash: Same procedure for methods A & B

1. Remove cover slip and wash slide twice in 0.5X SSC/0.1% SDS at 45°C for 5 min each.
2. Briefly rinse slide in distilled water.
3. Air dry.
4. Apply 20 µl DAPI/antifade (40 ng/ml) to the hybridized target and cover with cover slip (24x50 mm).

Procedure Note: Lower concentration of DAPI/antifade allows better signal visualization under triple band filter.

REAGENT PREPARATION

Note: Use autoclaved water for the preparation of all stock and working solutions

Alcohol Series

•Prepare fresh 70%, 80% and 100% ethanol and store at room temperature (RT).
•Prepare a second set of ethanol series and store at -20°C for probe denaturation using the formamide method.

20% (w/v) Sodium Dodecyl Sulfate (SDS)

•Dissolve 1 g of SDS in distilled water to a final volume of 5 ml. This reagent may be stored at room temperature (RT). Resolubilize any crystallized SDS by heating at 37°C prior to use.

20xSSC

•Dissolve 175.3 g Sodium chloride and 88.23 g tri-sodium-citrate in 900 ml of water.
•Bring the volume to 1000 ml and adjust to pH 7.0 with hydrochloric acid (HCL). (Sigma Catalog # S6639).

70% Formamide/2XSSC

•For 50 ml, add 35 ml formamide, 10 ml distilled water, 5 ml 20X SSC. Adjust pH to 7.0 using HCL. Pre-heat to 75°C in a water bath. (Sigma Catalog # F7503).

0.5xSSC/0.1% SDS

•For 1000 ml mix 25 ml of 20X SSC and 5 ml of 20% SDS in sterile distilled water and store at RT.

0.01M HCL

•Add 0.5 ml of 1M HCL to 49.5 ml of sterile distilled water. Pre-warm the solution to 37°C in a water bath.

Pepsin Stock Solution

•Prepare 10% stock (100 mg/ml) in sterile distilled water. Store at -20°C. (Sigma Catalog # P6887.)

Phosphate Buffered Saline (PBS) (1x)

•Prepare PBS and store at RT. (Sigma Catalog # 1000-3).

1% Formaldehyde

•Add 2.7 ml of 37% formaldehyde to 100 ml of 1X PBS and store at RT. (37% Formaldehyde - Sigma Catalog # F1268)

2X SSC/0.1% Tween 20

•Add 100 ml of 20X SSC and 1.0ml Tween 20 to a total of 1000 ml distilled water mix well on a magnetic stir plate and store at RT.

DAPI

•(Vector Laboratories H-1200)

Mounting medium/antifade

•(Vector Laboratories H-1000)

Tween 20

•(Fisher Scientific: BP337-100)

The Universal Linkage System (ULS) technology is covered by an international patent family for the linkage of any label to bio-organic molecules, owned by KREATECH Biotechnology BV, The Netherlands. This product or the use of this product may be covered by one of more patents of KREATECH Biotechnology, BV, including, but not restricted to, the following: EP 0,539,466; US 5,580,990; US 5,714,327; US 5,985,566; US 6,133,138.

The probes and methods of using the probes to detect chromosomal rearrangements and/or deletions are covered in the patent application published under US20020192692A1 owned by Cancer Genetics, Inc., USA

Litt, M., White R.L. A highly polymorphic locus in human DNA revealed by cosmid-derived probes. Proc. Natl. Acad. Sci. USA 82(18): 6206-6210, 1985.

Donion, T.A., Lalonde M., Wyman A., Bruns G, Latt S.A. Isolation of molecular probes associated with the chromosome 15 instability in the Prader-Willi syndrome. Proc. Natl. Acad. Sci. USA 83(12): 4408-4412, 1986.

Bibliography

Filter Requirements in Fluorescence Microscope		
dGreen: Excitation and Emission 501± 5 and 529 ± 5		
Rhodamine: Excitation and Emission 550± 5 and 577 ± 5	Filters:	
Red: 515-560	Green	480/40
	Triple	420/30
		465/20
		530/30
		640/40

Corporate Office:
 Cancer Genetics
 321 Fortune Blvd.
 Milford, MA 01757



For more information visit us at:
www.cancergenetics.com
 or email us at:
probes@cancergenetics.com