CytognomiX

SC-FISH Probes

Product Description

This product is IN DEVELOPMENT and intended for RESEARCH PURPOSES ONLY. IT IS NOT INTENDED FOR DIAGNOSTIC APPLICATIONS and/or COMMERCIAL PURPOSES.

Each tube has 1μg labelled DNA product in 10μl water. It should be stored at -20°C.

Main Protocol

This protocol describes fluorescence *in situ* hybridization (FISH) and detection of biotin labelled or digoxigenin labelled single copy probe. (Knoll and Litchter)

Materials

Chemical Reagents

- For slide and probe denaturation and hybridization
  - 70% formamide 2xSSC (sodium saline citrate), 50ml
  - 70%, 80%, 90% and 100% ethanol solutions, 50ml each
  - Biotin labelled or digoxigenin labelled single copy probe (1μg labelled DNA in 10μl water), stored at -20°C
  - Hybridization solution 4xSSC, 2μg/ul BSA (bovine serum albumin), 20% dextran sulfate, stored at 4°C
  - Deionized formamide

- For post-hybridization wash, detection, post-detection wash and mounting
  - 50% formamide 2xSSC (37°C), 50ml
  - 2xSSC (37°C), 50ml
  - 1xSSC, 50ml per wash, 3 washes
  - NeutrAvidin, Dylight™ 549 Conjugated (Thermo Scientific, Product Number 22837) for detecting biotin labelled probe
  - Cy™3-conjugated IgG Fraction Monoclonal Mouse Anti-Digoxin (Jackson ImmunoResearch Laboratories, Inc. Code Number: 200-162-156) for detecting digoxigenin labelled probe
  - Detector diluent: 4xSSC 1% BSA 0.02% Triton X-100, stored at 4°C
  - 1xSSC 0.1% Triton, 50ml
  - DAPI (4', 6-diamidino-2'-phenylindole, dihydrochloride) working solution 0.1μg/ml
  - McIlvaine's Buffer (pH 7.0)
  - Anti-fade (1,4-phenylenediamine dihydrochloride)

Other Supplies

- Coplin Jars
- Parafilm
- Plastic coverslips (22x22mm)
- Glass coverslips (22x22mm No. 0)
- Plexiglass
- Timer
- Ice
- Ruler
- Scissors
- Rubber cement
- Nail polish

Equipments

- Circulating water bath, set to 72°C
- Heating block, set to 72°C
- Warmed water bath at 37°C
- Incubator at 37°C
- Shaker platform

Procedure

- Turn on circulating waterbath 30 minutes before the experiment, set temperature to 72°C, warm up 70% formamide solution to 70°C and check temperature with a thermometer.
- Turn on heating block in advance and set temperature to 72°C.
- Warm up hybridization solution in 37°C water bath for 10 minutes, mix well by pipetting, return it to water bath till ready to use.

I. Denature Chromosomes

- Chromosome slide can be pre-treated with RNase and pepsin to digest away cytoplasm on the slide, but it is not a requirement for using sc probe. (See optional protocol 1)
- Denature slide in 70% formamide (70°C) for 2 minutes, then transfer slide to room temperature ethanol solutions in the order of 70%, 80%, 90% and 100% for 2 minutes each. Let air dry.

II. Denature probe

This procedure describes hybridization of 250ng probe on one slide, scale up reagent volumes accordingly to denature probe for hybridization to multiple slides. If a single copy probe gives high background noises, see Troubleshooting.

- To 2.5μl probe (250ng) add 10μl formamide, denature at 72°C for 5 minutes on a heating block and snap chill on ice.
- Add 10μl hybridization solution to denatured probe and formamide mixture, mix well and pipette the entire 22.5μl volume to the centre of hybridization area on slide, cover with a plastic coverslip.
- Seal the edges of coverslip with rubber cement, embed slide in Parafilm sandwich laid on a piece of Plexiglass and incubate 16 hours in 37°C incubator.

III. Post-Hybridization Wash and Detection

- Disassemble Parafilm sandwich, carefully remove coverslip from slide and immediately submerge slide in 50% formamide 2xSSC (37°C). Wash slide in 50% formamide solution for 30 minutes, agitate the Coplin Jar every 10 minutes.
- Transfer slide to 2xSSC (37°C) and wash for 30 minutes with agitation every 10 minutes.
- Transfer slide to 1xSSC (room temperature) and wash for 30 minutes with agitation every 10 minutes.
- Make 1:200 dilution of NeutrAvidin, Dylight™ 549 Conjugate (Thermo Scientific) for biotin labelled probe or 1:200 dilution of Cy™3 anti-digoxin antibody for digoxingenin labelled probe using 4xSSC 1%BSA 0.02% Triton X-100 as diluent. Detection reagent is light sensitive, perform this and all subsequent steps in a dark environment.
- Remove slide from 1xSSC, add 50μl of 1:200 diluted detection reagent to the centre of hybridization area on slide, cover with a piece of Parafilm that is cut out to about 22x22mm size
- Assemble a Parafilm sandwich with slides embedded, incubate slides in 37°C incubator for 45 minutes.

IV. Post-Detection Wash

- Disassemble Parafilm sandwich, gently remove Parafilm coverslip with a pair of forceps, submerge slide in 1xSSC (room temperature) and wash 15 minutes on a shaker platform at 150rpm.
- In a similar manner, wash slide in 1xSSC 0.1% Triton X-100 for 15 minutes, then in 1xSSC for 15 minutes.
- Remove slide from 1xSSC, add 50μl DAPI working solution (0.1μg/ml) to hybridized area on slide, cover with a piece of Parafilm coverslip and incubate in dark for 20 minutes.
- Gently remove Parafilm coverslip and rinse slide in McIlvaine's Buffer (pH 7.0) with agitation for 2 minutes. Remove slide from solution, tap off any liquid and let dry.
- Add 5μl anti-fade to the centre of hybridized area on slide, cover with a glass coverslip. Wait for a few minutes till anti-fade has evenly spread out underneath the coverslip, carefully push out any air if necessary.
- Seal the edges of coverslip with nail polish. Slide is ready for viewing or storage in –20°C freezer.

Optional Protocol 1

Limited RNase and pepsin slide pre-treatment removes cytoplasm that attracts non-specific bindings of probe. Prolonged slide pre-treatment with RNase and pepsin adversely affects chromosome morphology that may lead to low hybridization efficiency and indistinctive DAPI banding pattern. The following

procedure (adapted from Henegariu et al.) serves as a guide only, it is not a requirement for using single copy FISH probe.

Reagents

- RNase A working solution (0.5mg/ml) made by diluting stock RNase A (10mg/ml) with 2xSSC
- 2xSSC, 50ml
- 0.005% pepsin 0.01N HCl (50ml) prepared by adding 25µl 10% pepsin to 50ml warmed 0.01N HCl solution (37°C)
- 1xPBS (phosphate buffered saline), 50ml.
- 1xPBS 0.05M $MgCl_2$, 50ml.
- 70%, 90%, and 100% ethanol solutions, 50ml each.

Procedure

- Add 100µl RNase A working solution (0.5mg/ml) to hybridization area of slide, cover with Parafilm coverslip and incubate at 37°C for 15 minutes.
- Remove Parafilm coverslip, rinse slide in 2xSSC for 5 minutes with agitation.
- Incubate slide in 0.005% pepsin solution (37°C) for 5 minutes.
- Rinse slide in 1xPBS for 10 minutes, then in 1xPBS 0.05M $MgCl_2$ for 5 minutes on a shaker platform (150rpm).
- Wash slide in ethanol solutions in the order of 70%, 90% and 100% for 2 minutes each. Let air dry.

Troubleshooting

Hybridization of single copy probes that have not been validated by Cytognomix can sometimes have high background characterized by bright fluorescence painting along chromosomes, aggregating over nuclei, or bright signals distributing evenly on hybridization area, due to non-specific hybridization of repetitive elements that co-purify with single copy sequence during probe preparation. These bright noises can be seen at low power (10x objective) under the fluorescent microscope and are suppressed by adding COT-1 DNA.

Additional Reagent

- COT Human DNA (Roche Applied Sciences, Cat No. 11581074001)

Additional Equipment

- Speedvac system

Procedure

- Add 8-10μg COT-1 DNA (1μg/μl) to 250ng probe (2.5μl) in a microfuge tube, lyophilize in a Speedvac Centrifuge for 25 minutes or longer until the final volume is 3-4μl.
- Add 10μl formamide to probe/Cot-1 DNA mixture, denature on a heating block at 72°C for 5 minutes, snap chill on ice
- Add 10μl of warmed and well-mixed hybridization solution,
- Place probe mixture in 37°C incubator for an hour before using on a slide as described in main protocol

  (Preannealing of probe with COT-1 DNA blocks repetitive sequences from binding non-specifically to chromosomes and to elsewhere on slide)

References

1. Knoll, J.H.M. and Lichter, P. 1994. In situ hybridization to metaphase chromosomes interphase nuclei. In *Current protocols in human genetics* Vol. 1, unit 4.3. (eds. N.C. Dracopoli, et al), John Wiley, New York

2. Henegariu O, Heerema NA, Lowe Wright L, Bray-Ward P, Ward DC, et al. Improvements in cytogenetic slide preparation: controlled chromosome spreading, chemical aging and gradual denaturing. *Cytometry*. 2001;43:101-109.

Contact Information

Further information on Cytognomix Inc.'s offering of single copy products is available on www.cytognomix.com.

Cytognomix Inc.
Stiller Centre for Technology Commercialization
700 Collip Circle #150
London, ON N6G 4X8
Canada

Phone: 519-858-5050
Fax: 519-858-5142
E-mail: info@cytognomix.com