# DNA SPECTRAL KARYOTYPING HYBRIDIZATION & DETECTION PROTOCOL

For Research Use Only - Not for use in Clinical Diagnosis

The DNA Spectral Karyotyping Reagents are designed to enable simultaneous visualization of all chromosomes in different colors. The distinction between the dyes can be performed only with the SKY® spectral imaging system from Applied Spectral Imaging.

The following procedure is intended for hybridization of the Spectral Karyotyping Reagents on a normal metaphase slide preparation. Slide quality is one of the most important factors affecting the degree of hybridization. It is highly recommended that sample slides are viewed under phase contrast before, during and after pretreatment steps to ensure a successful hybridization. Sample slides that are sparse, have visible cytoplasm surrounding the metaphase spreads, or were aged at room temperature for more than 2 weeks are not recommended for use. For long term storage, dehydrate and store slides with a desiccant at -20°C, or store the cells in fixative at -20°C and drop slides 1-3 days before hybridization.

**Reagents-supplied by ASI:**

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|---|---|---|
| Vial 1 | SpectralKaryotyping(**Human/Mouse/Rat**) Reagent | 10µl/slide |
| Vial 2 | Blocking Reagent | 100µl/slide |
| Vial 5 | Anti-fade-DAPI Reagent | 20µl/slide |

**STORE ALL REAGENTS AT +4$^0$C**

**Reagents Required/ Not Supplied:**

- Cy5 staining reagent (prepare from Vial 3 from Applied Spectral Imaging's CAD kit Cat#: CAD03 or prepare according to Appendix A)
- Cy5.5 staining reagent (prepare from Vial 4 from Applied Spectral Imaging's CAD kit Cat#: CAD04 or prepare according to Appendix A)
- 20XSSC (prepare 1XSSC, 2XSSC, 4XSSC)
- Distilled water
- Formamide (molecular biology grade) [Sigma, Cat#: F7503]
- 70% , 80%, 100% Ethanol
- Tween 20 [Sigma, Cat#: P-9416]

REAGENT PREPARATION:

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***DAY 1***

| | |
|---|---|
| 1. Ethanol series | Prepare 70%, 80% and 100% ethanol series, place the 70% at -20°C and the 80% and 100% at room temp |
| Denaturation solution | Add 35 ml formamide, 10 ml distilled H2O, 5ml 20X SSC (final concentration is 70% formamide/2X SSC). Adjust pH to 7.0 using HCL, heat to 72°C. |

***Day 2***

| | |
|---|---|
| Rapid wash (0.4 X SSC solutions) | Add 1 ml 20X SSC<br>49 ml distilled water<br>**Total: 50 ml**<br>Mix well and heat to 74°C. |
| Washing solution III (4 X SSC/0.1%Tween 20) | Add 100 ml 20X SSC<br>400 ml distilled water<br>0.5ml Tween 20<br>**Total: 500 ml** |

Caution: Always wear gloves and safety glasses when working with any reagents and chemicals. Follow all laboratory safety guidelines when using this procedure.

# Protocol

***Please note that the hybridization time for Spectral karyotyping Reagents is 24-36hrs.**

## DAY 1

### *A) Trypsin Treatment*

Prepare and select samples for hybridization. Look at slides under phase and note cytoplasm. Select the best area of the slide and mark it**.** **<u>If no cytoplasm is observed and the slides look clean, continue with the denaturation step.</u>**

1. Wash slides briefly in Earl's medium.
2. Put 0.2-0.4ml of Trypsin/EDTA (5g/l Trypsin&2gr/l EDTA) in 50 ml Earl's medium at RT.
3. Incubate the slides for 20-40 seconds in the Trypsin solution.
4. Wash in water and dehedrate in ethanol series: 70%, 80% and 100% for 2 minutes each wash.
5. Air-dry the slides and continue with denaturation.

### *B) Chromosome denaturation*

1. Put the slides in 2XSSC at RT for 2 min and then dehydrate in Ethanol series: 70%, 80% and 100%, 2 min. each. Air dry.
2. Heat 40ml of denaturation solution to 72°C (±2°C) in a glass Coplin jar. Place slides in the solution for 1.5 minutes. **DO NOT OVERDENATURE**, some samples denature in 60 seconds. Slide warmer can also be used for denaturation: put 100µl of the denturation solution on the slide, cover with a cover glass and put on a slide warmer at 74°C for 1.5 minutes.
3. Immediately place slides in **Cold** 70%, 80% and 100% ethanol, 2 minutes each. Air-dry.

### *C) Probe denaturation and hybridization*

1. Centrifuge briefly the content of the Spectral karyotyping Reagent **<u>(vial # 1 supplied by ASI).</u>**
   **Note: Some red precipitation or clumps may normally be visible in this vial**
2. Mix well the content of the vial, including the red precipitation, by pipeting up and down for several times. Take 10µl for each slide, put in an Ependorf tube and denature the probe by incubation at 80°C in a water bath for 7 minutes.
3. Put in a water bath at 37°C for 10 minutes.
4. Add 10µl from the denature Spectral karyotyping Reagent to the denaturized chromosome preparation.
5. Place an 18 x 18mm$^2$ glass cover slip over the probe mix, being careful not to trap air bubbles under the cover slip. Seal the edges with rubber cement. Transfer the slide to a humidified chamber or container and place in incubator or baking oven set at 37°C for 24-36 hours.

## DAY 2

1. Remove slides from the humidified chamber and carefully remove the rubber cement.
2. Put slides in a Coplin jar containing rapid washing solution (0.4XSSC) at 72°C (±2°C) for 2–5 min.
3. Dip slides in washing solution III (4XSSC/ 0.1%. Tween 20) for 1 min.

   **Optional step: Apply 80µl of blocking reagent (vial # 2 - supplied by ASI), place a plastic cover slip (24X60m m2) and incubate at 37°C for 30 min.**
4. Tilt slides and allow fluid to drain. Apply 80µl of Cy5 Staining Reagent (vial # 3 -supplied by ASI). Place a plastic cover slip (24X60mm2) and incubate at 37°C for 40 minutes.
5. Wash slides 3 times in washing solution III (4XSSC/0.1% Tween 20) at 45°C for 2 minutes each wash in a water bath.
6. Apply 80µl of Cy5.5 Staining Reagent (vial # 4 -supplied by ASI), place a plastic cover slip (24X60mm2) and incubate at 37°C for 40 minutes.
7. Repeat step 5.
8. Tilt slides and allow fluid to drain. Put 20µl from the Anti-fade-DAPI Reagent (vial # 5 - supplied by ASI); place a cover glass (24X60mm2) over the surface. Try to remove any air bubbles that may have formed.
9. **The slides are now ready for imaging with the HiSKYV spectral imaging system from Applied Spectral Imaging.**

# APPENDIX A

## Preparation of Cy5 staining reagent (Vial #3) and Cy5.5 staining reagent (Vial #4)

### *MATERIALS*

- Anti digoxin (Sigma, D8156) 0.1ml.
- Cy5 StrepAvidin (Rockland, S000-06 or Amersham, PA45001) 1mg. Stock solution:1mg/ml, dissolved the content of the bottle in 1 ml sterile water, store in small aliquots in -20° C
- Cy5.5 sheep anti mouse (Rockland, 610-113-121) 1mg. Stock solution: 1mg/ml, dissolve the content of the bottle in 1 ml sterile water, store in small aliquots in -20° C

### *PROCEDURE*

***Vial No. 3:***

- Take 1 ml of 4XSSC, add 5µl of anti Digoxin and 5µl of Cy 5 Strep Avidin.

***Vial No. 4:***

- Take 1 ml of 4XSSC, add 5µl of Cy5.5 anti mouse.
- Use the diluted vials 3 and 4 according to the regular SKY protocol.
- Discard the diluted antibodies at the end of the day.
- Should longer storage of the diluted vials 3 and 4 be needed, add 1% of BSA fraction V (Roche 735078 or for USA only: Roche 100062) to the 4XSSC solution, e.g. Add 0.1gr BSA to pre warmed (37◦C) 10 ml 4XSSC. Vortex well and leave at RT until dissolved. Store at 4◦C.
- When using this buffer, the diluted vials 3 and 4 will stay stable for several days up to a month.