Cell Slide Preparation for FISH

Materials/Equipment:

Cells: cultured cells or other cell samples

Solution I: 0.4% KCL. Autoclaved and stored at 4°C,

Solution II: 0.8% sodium citrate. Autoclaved and stored at 4°C,

Fixatives: Methanol:Acetic acid = 3:1, freshly made.

Equipment:

- Glass slides
- Slide warmer
- Microscope

Procedures:

1.1.1 Harvest 100,000-500,000 cells into 15-mL centrifuge tube,

1.1.2 Centrifuge at 1,000x g for 5 min. to pellet the cells,

1.1.3 Discard most of the medium and leave about 0.2 ml.

1.1.4 Gently re-suspend the cell pellet in the 0.2 mL medium

1.1.5 Add 10 mL of 1:1 mixture of **0.4% KCl**:**0.8% sodium citrate** drop-wise

1.1.6 Mix gently by inverting the tubes a few times.

1.1.7 Incubate in a 37°C water bath for 20 min.

1.1.8 Add 0.1mL of freshly made **Fixatives** (3:1 of Methanol:Acetic acid)

1.1.9 Mix gently by inverting the tubes a few times.

1.1.10 Collect the cells by centrifuge at 1,000x g for 5 min.

1.1.11 Discard most of the supernatant and leave about 0.1 ml.

1.1.12 Re-suspend the pellet by tapping the bottom

1.1.13 Add 5 mL of **Fixatives** drop-wise; mix well,

1.1.14 Let stand on ice for 20 min.

1.1.15 Spin at 3,000x g for 5 min. to pellet the cells, and discard the supernatant

1.1.16 Re-suspend the cell pellet thoroughly in 5 ml of ice-cold **Fixatives**

1.1.17 Spin at 3,000x g for 5 min. to pellet the cells, and discard the supernatant

1.1.18 Repeat steps 16 and 17 two additional times.

1.1.19 Re-suspend the cell pellet in 1 ml of ice-cold **Fixatives;** place on ice until the next step.

Note: Cells in **Fixatives** can be stored at -20°C for up to one year.

1.1.20 To prepare the cell slides, spin at 3,000x g for 5 min. to pellet the cells, and discard the supernatant,

1.1.21 Re-suspend the cells thoroughly in 1mL of freshly made ice-cold **Fixatives**

1.1.22 Drop 5-10ul of the cell suspension on a glass slide at room temperature.

1.1.23 Allow samples to air dry.

1.1.24 Examine slide under a light microscope using a 20X objective.

1.1.25 ~50-200 cells visible in one field will be the recommended cell density (i.e., 50-200 cells per field)

- If cell density is too low, apply another 5-10 µl of cell suspension on the circle. Allow sample to dry and examine under light microscope. Repeat if necessary.
- If cell density is too high, dilute the cell suspension sample with ice-cold **Fixatives** and repeat steps 22-25 on new glass slides

1.1.26 Prepare 2-3 additional slides by repeating steps 22-25.

1.1.27 Bake slides at 56°C overnight.

1.1.28 Use the slides for FISH.

1.1.29 Store additional slide(s) at -20°C in 100% ethanol.

Note: Fixed slides are stable at -20°C for up to one year. Store any remaining cell suspension at -20°C for up to one year in the event preparation of additional slides is necessary.