

## Mitotic Chromosome Spreads from Mammalian Cells

### Reagents:

Karyomax Solution from Gibco

Hypotonic Saline (0.075M KCl) in dH<sub>2</sub>O

Methanol:Acetic Acid 3:1

### Protocol:

#### -Karyomax Treatment:

(1) Treat adherent cells culture with Karyomax Solution (1:100 in 10mL of Culture in 10cm dish), incubate in T.C. incubator for 30-60 minutes.

#### -Cell Fixation:

(2) Rinse plate with 1x PBS, scrape cells into 1xPBS in a 15ml Conical, pellet gently (900rpm, 5 minutes at RT), remove all but ~400ul of 1xPBS buffer

(3) Dropwise add, 2-3 ml of 37° C Hypotonic Saline to the top of the slurry, mix by gentle swirling.

(4) Incubate at 37° C for 15-20 minutes.

(5) Centrifuge as above.

(6) Remove supernatant without disturbing pellet, quickly but gently add Methanol:Acetic Acid fixative down the side of the 15ml Conical. Allow cells to incubate on the benchtop in this solution for 30min.

-Alternatively, cell solution can be stored at 4°C at this point.

(7) After incubation, centrifuge cells, remove supernatant. Resuspend in new fixative, using the same technique as described above. Repeat 2x.

(8) After repeated fixation steps, resuspend the pellet in just enough fixative to make a thin cell suspension. The actual concentration is variable and depends upon preference, but should be slightly opaque and more viscous than the fixative itself.

#### Slide Preparation:

(9) Prior to use, immerse pre-cleaned slides in fixative, allow to dry completely, facilitate drying with lint-free tissue if necessary.

(10) Using a PFE Dropper, collect some cell suspension and drop onto a slide positioned at ~30°-45° angle from a height of 1M.

(11) Dropper various numbers of drops onto different slides in an attempt to optimize concentration of cells on the slide. Aim for the middle of the slide.

(12) As slide begins to dry, you can dry using compressed air from a can, or air from the lab supply hosing. **Rapid drying is critical for proper spreading of metaphase chromosomes.**

(13) Drop a single drop of ~20uL ProLong Gold Antifade Reagent + DAPI directly onto slide. Using a pre-cleaned glass coverslip, drop gently over the ProLong Drop, wait until it spreads out fully.

(14) Store in -20°C in a dark box until ready to examine on Fluorescent Scope.