Protocol

This procedure describes proper methodology for preparing cell culture samples for KromaTiD G-Banding assay.

Please Note:

- Materials or Reagents with specific vendor & catalog numbers are considered essential to the protocol.
- Do not use expired reagents or materials
- Critical steps and techniques are noted with bold, underlined text, and marked with a

MATERIALS AND EQUIPMENT

General Materials:

- Vented culture flasks (T-25 or larger)
- 15mL polystyrene (see Tech Note 1) conical tubes (VWR Cat# 21008-197 or equivalent)
- Serological pipettes
- Glass pasteur pipettes and bulb (optional, recommended for use in Step 13 for drop wise addition if serological pipet does not have a slow speed expel function)
- 50mL conical tubes
- 75mM Potassium Chloride (Fisher Cat# 10575090 or equivalent; can be made fresh but must be less than 7 days old if solution is made with dry KCl and molecular grade H20)
- Methanol-molecular grade (Fisher Cat# A412-4 or equivalent)
- Glacial Acetic Acid (Fisher Cat# A38C-212 or equivalent)
- 70% ethanol
- Decon BDD Bacdown Detergent Disinfectant (Fisher Cat# 04-355-13) or equivalent
- Screw cap cryovials
- Parafilm

Cell Culture Materials:

- Growth media specific for the cell type in culture
- Colcemid (KaryoMax, Gibco Cat# 15210-040 or equivalent)

Equipment:

- P20, P200 and P1000 pipettes
- Certified laboratory biosafety cabinet (suitable for tissue culture)
- CO2 cell culture incubator
- Serological Pipettor

****Tech Note 1: Polypropelene tubes are not resistant to fixative, which can result in cracking and loss of sample as well as plastic degradation products present in the sample.*

PROCEDURE

Preparation of Cells:

- Prepare at least 1 flask (T-25 or larger) per sample to be tested.
- The culture should be passaged when the appropriate confluence for the cell or colonies has been reached.
- For induced pluripotent stem cells (iPSC), cells should be seeded into the appropriately coated vessel and can be prepared with or without a feeder layer. Cells to be harvested should be split 1:3 into at least one T-25 flask or 3 wells of a 6 well plate per sample.

Note: KromaTiD is happy to review and provide guidance on an experimental plan appropriate for your specific cell culture

Colcemid Addition and Harvest:

1. Incubate in temperature and CO2 conditions appropriate for the culture until the cells ready for colcemid block (see table below)

Cell Type	Add colcemid
Non-adherent cell lines	24 Hours after splitting
Adherent cell lines	When cells are 75% confluent
CD34 cells	At time of splitting or thawing (if after thawing, allow a 24-48 hour rest period for cells to recover)
iPSC	When distinct colonies begin to grow and cells are in logarithmic growth phase (typically 48-72 hrs after splitting depending on original cell concentration). Colonies should be visible to the eye.

2. Add colcemid at 0.1ug/mL or 10ul/mL and incubate per the table below.
    a. For adherent lines, cells can be visualized at 20-40X magnification on an inverted light microscope and will appear to be "rounding up" as the mitotic cells accumulate in the cytokinetic block.
    b. See table below for recommended colcemid incubation time per cell type.

Cell Type	Colcemid incubation time
Non-adherent cell lines	2 Hours
Adherent cell lines	3 Hours
CD34 cells	2 Hours
iPSC	2 Hours

3. After colcemid incubation, transfer the medium to a 15mL polystyrene conical.
    a. For adherent cells, trypsinize the culture according to the protocol specified for the cell type and transfer the cells to the conical containing the media.
    b. For iPSC, harvest cells according to the protocol specified for the cell line (such as 0.5mM EDTA). After removing EDTA, rinse plate with appropriate iPSC media and transfer to a 15 mL polystyrene conical. If a 6 well plate is used, combine the contents of each well per sample after the cells have been removed from the plate.
4. Centrifuge the samples at 1000rpm for 10 minutes at room temperature.
    a. ⚠ **Aspirate off medium with care to leave 0.5 mL-1.0 mL media in the tube with the cell pellet.**
    b. ⚠ **For larger cell pellets leave 1.0 mL in the tube.**
5. Thoroughly suspend cell pellets by vigorously flicking the conical with finger or flicking against palm of hand.
6. Add 7mL of prewarmed (37 C) 75mM KCl hypotonic solution and incubate 30 minutes at 37 C, inverting every 5 minutes.
    a. For iPSC: pipet up and down to break up clumps into single cells. Some clumps may persist.
7. Add 1.5mL freshly prepared 3:1 methanol to acetic acid fixative to each conical and invert several times.
8. Centrifuge samples at 1000rpm for 10 minutes at room temperature.
9. ⚠ **Aspirate supernatant (do not pour off), leaving 0.5mL-1.0 mL with the cell pellet.**
    a. For larger pellets leave 1.0 mL in the tube.
10. ⚠ **Thoroughly suspend the cell pellet with finger flicking or flicking against palm of hand.**
11. While vortexing at low speeds, add 5mL 3:1 methanol to acetic acid fixative.
    a. ⚠ **Add the first 2mL dropwise slowly to minimize clumping.**
    b. ⚠ **A serological pipettor with slow speed expel functionality (loaded with a 2-5mL pipet) or a glass pasteur pipet and bulb is recommended for drop-wise addition.**
12. Fix at room temperature for 20 minutes.
    a. At this point you may place the conicals at -20°C until ready to do the remainder of the fixative washes.
13. Centrifuge sample at 1000rpm for 10 minutes.
14. Aspirate off all fixative, leaving about 0.5mL covering the cell pellets.
15. ⚠ **Thoroughly suspend the cell pellet by gently flicking the bottom of the conical tube.**
16. Add 5mL fixative while vortexing at low speeds, then centrifuge at 1000rpm for 10 minutes.
17. Repeat steps 16-18 two additional times.

Shipping Instructions:

This procedure describes the proper methodology for shipping fixed cell pellets to KromaTiD for G-Banding analysis.

Interior Container:

1. Remove the majority of the fixative after Step 17, leaving 1mL on the cell pellet.
2. Flick the cell pellet into solution thoroughly and transfer full volume to a 2.0 mL screw top cryovial.
3. Use 0.5mL fresh fixative to rinse out 15mL conical tube in order to collect any cells on the walls of the tube and transfer to the cryovial for a total volume of 1.5-1.7mL cells in fixative.
4. Tighten cap and seal with parafilm.

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Intermediate:

1. Wrap each vial in absorbent paper toweling/absorbent sleeve and place in a sealable plastic bag.
2. Put into a padded cardboard box with a cool gel pack.
3. Include requisition/information on each specimen.

Exterior:

1. Box can be shipped in a FedEx “Clinical Pak”.
2. **Shipping should be addressed to:**
   1880 Industrial Circle, Suite A, Longmont CO, 80501

Full Description of Goods:

- Nonviable cells in up to 6mL 3:1 methanol acetic acid for research use only
- Non-infectious, non-hazardous, non-toxic
- Dangerous goods in expected quantities

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