RUO
RESEARCH USE ONLY

Instructions For Use

Cell Culture Protocol for use in KromaTiD G-Banding Assay

Reagents Required

- Demecolcine solution, 10 µg/mL (pseudonym Colcemid™) - KromaTiD Cat. No. COL-001, -002, -003; or equivalent)
- 75 mM potassium chloride (KCl) (Fisher Cat# 10575090 or equivalent)
- Methanol - molecular grade (Fisher Cat# A412-4 or equivalent)
- Glacial acetic acid (Fisher Cat# A38C-212 or equivalent)
- 70% ethanol (VWR Cat#EM-4450S or equivalent)
- Decon BDD Bacdown Detergent Disinfectant (Fisher Cat# 04-355-13 or equivalent)
- Growth media specific for the cell line in culture

Equipment and Supplies

- Certified biosafety cabinet or equivalent.
- Appropriate culture vessels
- Serological pipettor & pipets
- 50 mL conical tubes
- ⚠ 15 mL *polystyrene* conical tubes (VWR Cat# 21008-197 or equivalent)
  » WARNING: DO NOT USE POLYPROPYLENE TUBES. *Polypropylene tubes are **NOT** resistant to the fixative and will leak and will contaminate the sample.*
- 2.0 mL screw cap cryovials
- Parafilm

G-Banding Assay Cell Culture Protocol

Cell Preparation Procedure

- Prepare at least $1x10^6$ cells per test sample.
- Pass the culture when the appropriate confluence for the cell line or colonies has been reached.
- For iPSC, seed into appropriately coated vessel with or without 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.*

Demecolcine (Colcemid™) Addition and Harvest

1. Warm 75mM potassium chloride (KCl) solution in 37°C water bath.
2. Add 10 µL of the 10 µg/mL KromaTiD demecolcine solution (Colcemid™) per mL of media directly to each culture vessel for a final concentration of 0.1 µg/mL. The table below provides guidance on timing of demecolcine addition and length of incubation.

Cell Type	Suggested Timing for Demecolcine Addition	Incubation Time
Non-adherent cell lines	24 hours after splitting	2 hours
Adherent cell lines	When cells are 75% confluent	3 hours
CD34 cells	At time of splitting	2 hours
iPSC	When distinct colonies begin to grow (typically 48–72 hours after splitting)	2 hours

3. At the end of the incubation period, harvest cells.
    a. Dissociate adherent cells and transfer the cells to a polystyrene conical.
    b. For suspension cells, transfer the cells directly to a polystyrene conical.
4. Centrifuge the samples at room temperature, 1000 rpm for 5 minutes.
5. Carefully aspirate supernatant, leaving 0.5 mL to 1mL of media behind with the cell pellet.
6. Thoroughly resuspend cell pellets by pipetting up and down with a P1000. Be sure that all cell clumps have been dispersed.
7. Add 10mL of 37°C 75mM KCl hypotonic solution, cap tube and invert several times.
    a. For iPSC, pipet up and down to break up clumps into single cells. Some cell clumps may persist.
8. Incubate for 25 minutes at 37°C.
9. Add 1.5 mL of freshly prepared 3:1 methanol to glacial acetic acid fixative to each 15 mL polystyrene conical and invert gently several times.
10. Centrifuge the samples at 1000 rpm for 10 minutes at room temperature.
11. Carefully aspirate supernatant, leaving 0.5 mL to 1.0 mL of media behind with the cell pellet.
12. Thoroughly resuspend cell pellets by pipetting up and down with a P1000. Be sure that all cell clumps have been well dispersed.
13. Add 2 mL of 3:1 methanol to glacial acetic acid fixative dropwise with gentle mixing to minimize clumping, followed by an additional 3 mL of fixative. Cap tubes and invert several times to mix.
14. Leave at room temperature for 20 minutes.

» **NOTE** *Sample tubes can be held at -20ºC overnight after Step 14.*

15. Centrifuge the samples at 1000 rpm for 10 minutes at room temperature.

    » **NOTE** *Some cell types (especially iPSCs and T-Cells will have a translucent cell pellet that is difficult to visualize after exposure to fixative. Take care not to aspirate the pellet while removing the supernatant after each fixative wash.*

16. Aspirate the supernatant with care to leave 0.5 mL of media behind with the cell pellet.
17. Resuspend the cell pellet thoroughly by gently flicking the tubes.
18. Add 5 mL of the 3:1 methanol to glacial acetic acid to the cell pellet, cap the tubes and gently invert several times.
19. Centrifuge at 1000 rpm for 10 minutes at room temperature.
20. Repeat steps 16–19 two more times.
21. Aspirate the supernatant after centrifuging, leaving behind 1.0 mL to cover the pellet.
22. Gently flick the cell pellet into solution thoroughly and transfer the full volume to a 2 mL screw cap cryovial.
23. Use 0.5 mL of fresh fixative to rinse out the 15 mL conical tube to collect any cells on the walls of the tube and transfer to the cryovial for a total volume of 1.5–1.7 mL cells in fixative. Tighten the cap and seal with parafilm.
24. Please complete KromaTiD's sample submission form (available here). Send a digital copy to **samples@kromatid.com** and include a hard copy with the return shipment. Include any requisition information on this form.
25. Package the cell pellets for return to KromaTiD, see Shipping Instructions, next page.

Shipping Instructions

If shipping fixed cells to KromaTiD for dGH analysis, make sure vial top is tightly screwed shut and the top sealed with parafilm before packaging for shipment. Wrap each vial in absorbent paper toweling or absorbent sleeve and place in a sealable plastic bag. Place the sealed 2 mL cryovials in a padded cardboard box with a frozen gel pack. Include any requisition/information on the sample submission form. Box can be shipped in a FedEx "Clinical Pak".

Shipping address:

KromaTiD
1880 Industrial Circle, Suite A
Longmont, CO 80501

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