RUO
RESEARCH USE ONLY

Instructions For Use

Cell Culture Protocol for use in KromaTiD G-Banding Assay

Reagents Required

- Colcemid (KaryoMax, Gibco Cat#15210-040 or equivalent)
- 75mM Potassium Chloride (KCl) (Fisher Cat# 10575090 or equivalent; must be less than 7 days old if solution is made with dry KCl and molecular grade H_2O)
- 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
- Vented culture flasks (T-25 or larger)
- Serological pipettor & pipets
- 50mL conical tubes
- ⚠ 15mL *polystyrene* conical tubes (VWR Cat# 21008-197 or equivalent)

Note: *polypropylene tubes are not resistant to the fixative and will leak.*

- 2.0mL screw cap cryovials
- Parafilm

G-Banding Assay Cell Culture Protocol

Preparation of Cells

- Prepare at least 1 million cells per sample to be tested.
- The culture should be passaged when the appropriate confluence for the cell 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.*

Colcemid Addition and Harvest

Note: *37ºC pre-warmed 75mM KCl is required for Step 7.*

1. Incubate with normal cell culture conditions (temperature, %CO2, and humidity) until cells are ready for a colcemid block.

Cell Type	Colcemid Addition Notes	Colcemid Addition 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

2. Add colcemid at a final concentration of 0.1ug/mL by adding 10µL of colcemid per mL of culture media and incubate for indicated recommended time shown in the table above.
3. At the end of the colcemid blocking period, transfer the growth medium to a 15 or 50mL polystyrene conical, depending on culture volume.
    a. Dissociate adherent cells and transfer the cells to a polystyrene conical.
    b. For suspension cells, transfer the cells to a polystyrene conical.
4. Centrifuge the samples at room temperature at 1000rpm for 5 minutes.
5. Aspirate the supernatant with care to leave 0.5mL to 1mL of media in the tubes with the cell pellet.
6. Thoroughly resuspend the cell pellet by vigorously flicking the conicals.
7. Add 10mL of 37ºC 75mM KCl hypotonic solution and incubate for 25 minutes at 37ºC, inverting every five minutes.
    a. For iPSC, pipet up and down to break up clumps into single cells. Some clumps may persist.
8. Add 1.5mL of freshly prepared 3:1 methanol to glacial acetic acid fixative to each 15mL polystyrene conical and invert gently several times.
9. Centrifuge the samples at 1000rpm for 10 minutes at room temperature. The cell pellets should be subjectively 2-3X larger in size due to the swelling of cells in hypotonic solution.
10. Aspirate the supernatant with care to leave 0.5mL to 1mL in the tubes with the cell pellet.
11. Resuspend the cell pellet by gently flicking the conicals.
12. While vortexing at low-medium speeds, add 5mL of 3:1 methanol to glacial acetic acid fixative.
    a. Slowly add the first 2mL dropwise to minimize clumping.
13. Fix at room temperature for 20 minutes.
    a. At this point you may place the tubes at -20ºC until ready to do the remainder of the fixative washes.
14. Centrifuge the samples at 1000rpm for 10 minutes at room temperature.

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

15. Aspirate the fixative supernatant with care to leave 0.5mL in the tubes with the cell pellet.
16. Resuspend the cell pellet by gently flicking the conicals.
17. Add 5mL of the 3:1 methanol to glacial acetic acid to the cell pellet.
18. Centrifuge at 1000rpm for 10 minutes at room temperature.
19. Repeat steps 15 – 18.
20. Repeat steps 15 – 18 again.
21. Discard the supernatant after centrifuging, leaving behind 1.0mL to cover the pellet.
22. Gently flick the cell pellet into solution thoroughly and transfer the full volume to a 2mL screw cap cryovial. Use 0.5mL of fresh fixative to rinse out the 15mL conical tube to collect any cells on the walls of the tube and transfer to the cryovial for a total volume of 1.5-1.7mL cells in fixative. Tighten the cap and seal with parafilm.
23. Please complete KromaTiD's sample submission form and include a hard copy with the return shipment.
24. Package the cell pellets for return to KromaTiD, see Shipping Instructions below.

Shipping Instructions

If shipping fixed cells to KromaTiD for dGH analysis, make sure vial 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 2mL cyrovials 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 should be addressed to:

KromaTiD
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