RUO
RESEARCH USE ONLY

Instructions For Use

Cell Culture Protocol for Use in KromaTiD directional Genomic Hybridization™ (dGH™) Assays

Reagents & Materials Provided

- KromaTiD dGH Media Additive
- Return shipping materials included (shipping box & internal insulated box)
  - 2 mL screw cap cryovials
  - Return shipping label

Reagents Not Provided

- 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 – molecular grade (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
- Parafilm
- ⚠ 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, contaminating the sample.*

Handling & Storage of KromaTiD dGH Media Additive

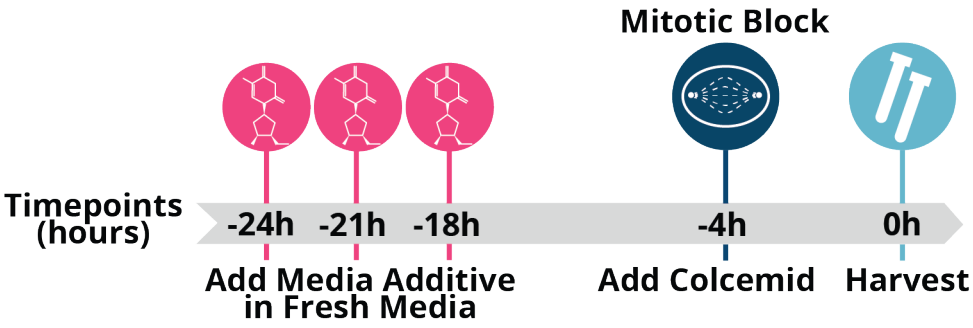
- Store media additive at -20ºC upon receiving.
- Media additive can be stored for up to 9 months at -20ºC or up to 30 days at 4ºC.
- ⚠ Once thawed, do not re-freeze.

Cell Culture Protocol for KromaTiD dGH Assays

24 Hours Before Harvest

1. Prepare media mixture for three culture timepoints by adding 1 µL of KromaTiD dGH Media Additive per mL of fresh culture media required.

» **NOTE** *Incubating cultures with dGH Media Additive starting at three different timepoints helps ensure capture of the best metaphase cells for analysis. Below is an example timeline which works well for most cell lines tested by KromaTiD.*



2. Adding fresh media with dGH Media Additive to cell cultures
    a. Suspension cell cultures
        i. Pellet cells at appropriate centrifuge speed at room temperature in conical tubes.
        ii. Aspirate supernatant and resuspend cell pellet.
        iii. Add fresh media with dGH Media Additive to cells, recap the tubes and gently invert several times.
        iv. Transfer cell suspension to a new culture container and return to incubator.
    b. Adherent cells
        i. When cell cultures are approximately 50% confluent, aspirate media and add the mixture of fresh media with Media Additive.
        ii. Return to incubator.

Day of Harvest

1. Warm 75 mM potassium chloride (KCl) solution to 37°C in water bath.
2. Directly add 10 µL of 10 µg/mL Demecolcine solution (Colcemid™) per mL of media to each culture flask, for a final concentration of 0.1 µg/mL.

» **NOTE** *Add Demecolcine directly to existing culture media. Do not replace current with fresh media before adding Demecolcine.*

3. Return to incubator for 4 hours.
4. At the end of the incubation period, transfer cells from their culture containers to 15 mL polystyrene conical tubes.
    a. Dissociate adherent cells before transferring.
    b. For suspension cells, transfer directly.
5. Centrifuge the samples at room temperature, 1000 rpm for 5 minutes.
6. Carefully aspirate supernatant, leaving 0.5 mL to 1 mL of media behind with the cell pellet.
7. Thoroughly resuspend cell pellets by pipetting up and down with a P1000. Be sure that all cell clumps have been dispersed.
8. Add 10 mL of 37ºC 75 mM KCl hypotonic solution, cap the tubes and gently invert several times.
9. Incubate for 25 minutes at 37ºC, inverting every 5 minutes.
10. Add 1.5 mL of freshly prepared 3:1 methanol to glacial acetic acid fixative to each 15 mL polystyrene conical tube. Cap the tubes and invert gently several times.
11. Centrifuge the samples at 1000 rpm for 10 minutes at room temperature.
12. Carefully aspirate supernatant, leaving 0.5 mL to 1 mL of media behind with the cell pellet.
13. Thoroughly resuspend cell pellets by pipetting up and down with a P1000. Be sure that all cell clumps have been dispersed.
14. 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.
15. Leave at room temperature for 20 minutes.

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

16. 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. In the following steps, take care not to aspirate the cell pellet while removing the supernatant after each fixative wash.*

17. Carefully aspirate supernatant, leaving 0.5 mL of media behind with the cell pellet.
18. Resuspend the cell pellet by flicking gently.
19. Add 5 mL of the 3:1 methanol to glacial acetic acid to the cell pellets, cap the tubes and gently invert several times.
20. Centrifuge at 1000 rpm for 10 minutes at room temperature.
21. Repeat steps 17–20 twice more.

    » **NOTE** *If shipping samples back to KromaTiD for dGH analysis, please follow the instructions below. Otherwise follow the dGH Assay Protocol that can be provided on request.*

22. Carefully aspirate supernatant, leaving behind 1.0 mL to cover the pellets.

23. Gently resuspend the cell pellets and transfer the full volume of individual samples into the 2 mL screw cap cryovials provided.
24. Use 0.5 mL of fresh fixative to rinse out the 15 mL conical tubes, collecting any cells on the walls of the tubes, and transfer to the cryovial for a total volume of 1.5–1.7 mL. Tighten the cap and seal with parafilm.
25. Label tubes clearly using a laboratory marker with chemical-resistant ink.
26. 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.
27. Package the cell pellets for return to KromaTiD following the shipping Instructions below.

Shipping Instructions

Make sure all vials are tightly screwed shut and the top sealed with parafilm before packaging for shipment. Place the sealed 2 mL cryovials in an insulated box with a frozen gel pack. Attach the return shipping label included with the original packaging.

Shipping address:

KromaTiD
1880 Industrial Circle, Suite A
Longmont, CO 80501

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