
dGH Cell Prep Kit Protocol

Purpose

This protocol provides guidance on cell culture and preparation prior to use in KromaTiD directional Genomic Hybridization (dGH™) assays.

Reagents & Materials Provided

- KromaTiD dGH™ Cell Prep Additive – KromaTiD Cat. No. DGH-0001, -0002, or -0003
- 2 mL screw cap cryovials (25 ct with DGH-0001; 50 ct with DGH-0002; 100 ct with DGH-0003)

Optional – Contact techsupport@kromatid.com for assistance in ordering these additional products.

- Box and gel packs for shipping cell samples to KromaTiD; Cat. No. KTID-003
- Shipping label (includes shipping provider charge), Cat. No. KTID-004

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. No. 10575090 or equivalent).
- Methanol - molecular grade (Fisher Cat. No. A412-4 or equivalent)
- Glacial acetic acid (Fisher Cat. No. A38C-212 or equivalent)
- 70% ethanol – molecular grade (VWR Cat. No. EM-4450S or equivalent)
- Decon BDD Bacdown Detergent Disinfectant (Fisher Cat. No. 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
- Laboratory benchtop centrifuge and appropriate swing bucket rotor
- 50mL 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 degrade and/or leak, compromising the sample.*

Handling & Storage of KromaTiD dGH Cell Prep Additive

- Store dGH Cell Prep Additive at 4°C upon receiving.
- dGH Cell Prep Additive can be stored up to expiry date at 4°C.

Important Notes Before Beginning

Purpose of dGH™ Cell Prep Additive

Cells for analysis in dGH assays undergo a specialized preparation method to enable the stripping of daughter strands from the metaphase chromosomes prior to dGH probe hybridization. This requires incorporation of the dGH™ Cell Prep Additive while cells are actively dividing and in S-phase.

Choosing dGH™ Cell Prep Additive timepoints

Incubating cultures with dGH Cell Prep Additive at three different timepoints helps ensure capture of sufficient correctly prepared metaphase cells for analysis. Determining the doubling time of your cell line or primary cell culture is essential for choosing the three timepoints to use.

The example timeline in Figure 1 is based on a cell line with a 24-hour doubling time. KromaTiD has found this timing generally works well for all white blood cell types, blood products and immortalized blood cell lines as well as non-cancerous fibroblast and epithelial cell lines. Cancer cell lines and other significantly modified cultures may need alternative planning. Please contact techsupport@kromatid.com if you have questions about how your cell timing could impact experimental planning.

Cell Number Requirements

To obtain sufficient metaphase chromosome spreads for dGH analysis, perform the experiments at a sufficient scale to yield a minimum of 1 million cells per timepoint sample at harvest.

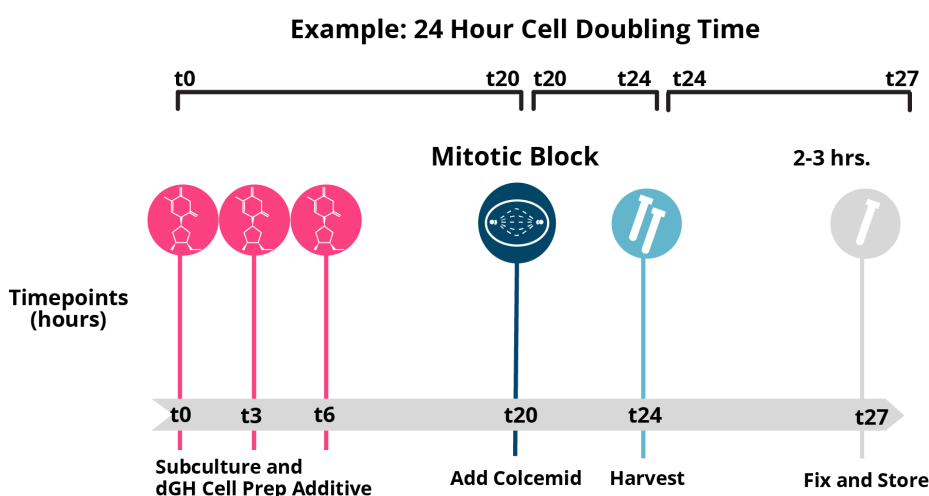


Figure 1: Example workflow for culturing cells with a 24-hour doubling time.

Sample Experimental Plan (Multiple Samples)

11/14 - 11/20

Samples:
■ = S1
■ = S2
■ = S3
■ = S4
■ = S5

Harvest Timepoints:
T1: 23 hours post analog addition
T2: 27 hours post analog addition

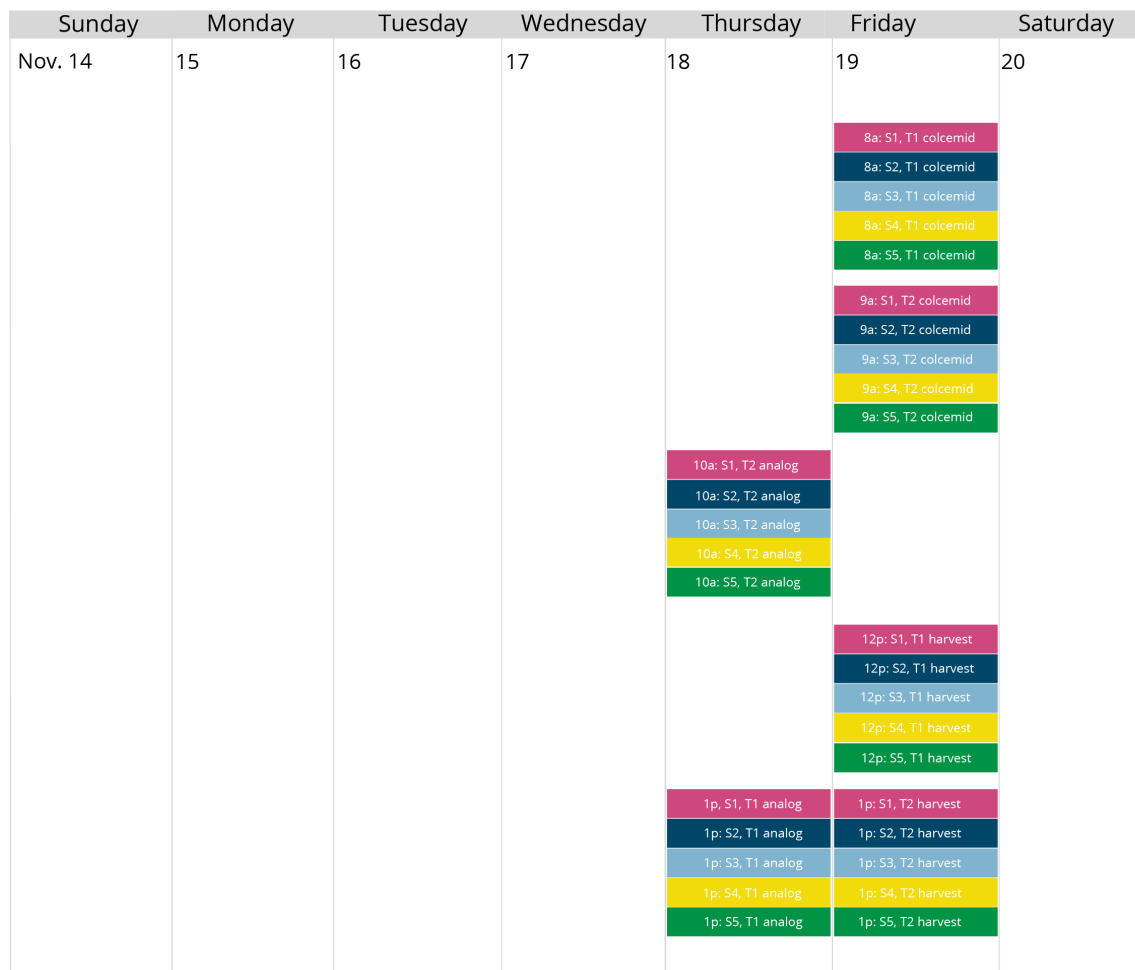


Figure 2: Example Experimental Plan (Multiple Samples). KromaTiD recommends processing cohorts of no more than 7 samples in parallel. Experiments with more than 7 samples should be planned with staggered cohorts, and separate harvest times.

Cell Culture Protocol for KromaTiD dGH Assays

24 Hours Before Harvest

1. Ensure that the total culture volume is sufficient to yield at least 1 million cells per timepoint at harvest. The cells can be cultured in flasks or multi-well plates and scaled to yield >1 million cells at harvest.
2. Adding fresh media with dGH Cell Prep 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 in fresh media, at a density that promotes culture expansion.
 - iii. Add 1 µL KromaTiD dGH Cell Prep Additive per 1 mL of culture volume.
 - iv. Return the culture to the incubator.
 - b. Adherent cell cultures
 - i. When the culture is 40–50% confluent, aspirate media and add fresh media.
 - ii. If the cells are more than 60% confluent, consider replating them to allow room for culture expansion.
 - iii. Add KromaTiD dGH Cell Prep Additive to the culture, using 1 µL of Additive per 1 mL of culture volume.
 - iv. Return the cultures to incubator.

Day of Harvest

1. Warm 75 mM potassium chloride (KCl) solution to 37°C in water bath and prepare fixative (3:1 Methanol: Glacial Acetic Acid).

Note: Fixative needs to be prepared for use within 24 hours and should always be prepared fresh each day of harvest.
2. Directly add 10 µL of 10 µg/mL demecolcine solution (Colcemid™) per mL of media to each culture vessel, 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 culture to incubator for 4 hours.
4. At the end of the incubation period, transfer the cells from their culture vessels to 15 mL polystyrene conical tubes.
 - a. Detach adherent cells thoroughly from vessels before transferring.

Note: Retain media supernatant prior to detachment (trypsinizing/ scraping) and use it to deactivate trypsin and/or rinse and collect the cells from the vessel prior to transferring to the 15 ml conical tube.

-
- b. For suspension cells, transfer directly. Multiple wells of sample (single timepoint) can be combined at this step. If media volume exceeds 15 mL, split into multiple 15 mL conical tubes, or use a 50 mL conical tube for this step and transfer volume to 15 mL polystyrene conical tube after step 7.
5. Centrifuge the samples at 180 g for 10 minutes at room temperature. If your centrifuge cannot be programmed using g, and you do not know the radius of your rotor, spin at 1000 RPM. A setting of 1000 rpm using a standard hanging-bucket style rotor centrifuge large enough to accommodate 15-50 mL conical tubes will achieve a sufficiently gentle g-force.
- Note:** Avoid centrifugation at higher g's. Cell pellets will over-clump during high-speed spins and cannot be disrupted sufficiently to provide exposure to the hypotonic in the following step. Please check the rotor size and calculate the RPM required to achieve 180g. G-force formula: $RCF (g \text{ force}) = \text{relative centrifugal force}$, $r = \text{rotational radius (cm)}$ $G \text{ Force (RCF)} = (RPM)^2 \times 1.118 \times 10^{-5} \times r$. RPM Formula: $RPM = \sqrt{[RCF / (r \times 1.118)] \times 1 \times 10^5}$.
- Note:** If your specific culture calls for a gentler, lower g-force spin than the one specified in this protocol, consult KromaTiD to determine appropriate modifications, and plan for a longer centrifugation time to accommodate the lower g-force.
6. Carefully aspirate supernatant, leaving 0.5 mL to 1 mL of media behind with the cell pellet.
7. Thoroughly resuspend cell pellets by vigorously flicking each conical tube, or by pipetting up and down.
- ▲ Be sure that all cell clumps have been thoroughly dispersed.
 - ▲ If a 50mL conical was required for steps 4-7, transfer the entire resuspended pellet to a 15mL polystyrene conical.
8. Add 10 mL of 37°C 75 mM KCl hypotonic solution, cap the tubes and gently invert several times.
9. Incubate for 30 minutes at 37°C, inverting every 10 minutes.
10. Add 1.5 mL of freshly prepared fixative to each 15 mL polystyrene conical tube. Cap the tubes and invert gently several times.
11. Centrifuge the samples at 180 g for 10 minutes at room temperature.
- ▲ This step involves centrifugation of swollen, sensitive cells. Centrifugation at higher g's may result in cell damage.
12. Carefully aspirate supernatant, leaving 0.5 mL to 1 mL of media behind with the cell pellet.
13. Thoroughly resuspend cell pellets by finger flicking the tube. CAUTION: Do not use a pipette to resuspend as the cells are fragile. Be sure that all cell clumps have been dispersed.
14. While vortexing at low-medium speed, add 2 mL fixative **dropwise** with gentle mixing to minimize clumping, followed by an additional 3 mL of fixative.
15. Leave the fixative suspension at room temperature for 20 minutes.
16. Centrifuge the samples at 180 g for 10 minutes at room temperature.
- Note:** At this point, sample tubes can be held at -20°C until ready to perform the remainder of the fixative washes.
17. Carefully aspirate supernatant, leaving 0.5 mL of media behind with the cell pellet.
-

Note: Some T cells, T cell lines and CAR-T cells may have a translucent or transparent cell pellet that is difficult to visualize after fixation. Take care not to aspirate the cell pellet while removing the supernatant after each fixative wash.

18. Resuspend the cell pellet by flicking gently.
19. Add 5 mL of fixative to the cell pellets, cap the tubes and gently invert several times.
20. Centrifuge at 180 g for 10 minutes at room temperature.
21. Repeat steps 17–20 twice more for a total of 3 washes in fixative.

Preparing samples for shipment to KromaTiD

22. Carefully aspirate supernatant, leaving behind 1.0 mL of fixative to cover the pellets.
23. Gently resuspend the cell pellets by flicking the vial, then 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.
25. Tighten the cap and seal with parafilm. Please ensure all tube labels are legible, and resistant to alcohol.
26. Please complete KromaTiD's Sample Submission Form (available upon request) and submit it to samples@kromatid.com. Include any requisition information on this form.
27. Place the sealed 2mL cyrovials in an appropriate sample box. Ship the sample box in an insulated shipper with appropriate cold packs (See *Notes on Sample Shipping* and example table below.)

Notes on Sample Shipping: Ship the samples with your preferred carrier using priority overnight service. Shipping using frozen gel packs will only guarantee temperature maintenance overnight (particularly in warmer months). Shipping using dry ice (5 to 8 lbs.) will guarantee temperature maintenance for two to three days (requires specific shipping procedures and the use of a UN 1845 label on the shipping box). Your shipping department will have this information. If you do not have a shipping department and require assistance, please reach out to us at the contact listed below.

KromaTiD offers the following products to expedite your sample shipment.

- Box and gel packs for shipping cell samples to KromaTiD, Cat. No. KTID-003
- Shipping label (includes shipping provider charge), Cat. No. KTID-004

Please contact sales@kromatid.com for pricing.

Shipping address

KromaTiD
1880 Industrial Circle, Suite A
Longmont, CO 80501

Example Shipping Containers:

Uline insulated shipping kits

Model No.	Inside Dimensions L x W x H	Wall Thickness	Outside Dimensions L x W x H	Sample Box L x W x H	# of Samples	Weight
S-7887	6 x 4 1/2 x 3"	1"	8 x 6 1/2 x 5"	3 x 3 x 2"	25	10 to 12 oz. (Cold Packs)
S-9903	8 x 6 x 4 1/4"	1 1/2"	11 x 9 x 7 1/4"	5 1/4 x 5 1/4 x 2"	81	32 to 48 oz. (Cold Packs)
S-7359	8 x 6 x 9"	1 1/2"	11 x 9 x 12"	5 1/4 x 5 1/4 x 2"	81	5 to 8 lbs. (Dry Ice)

Sample box dimensions are for paperboard boxes

Customer Notification

1. All Products and Deliverables are supplied for internal scientific research purposes only and are not intended for i) human consumption, including, but not limited to, foods or pharmaceuticals, ii) diagnostic purpose including, but not limited to, human or veterinary *in vivo* or *in vitro* diagnostics, or use in cosmetics or other goods. Research purposes means *in vitro* laboratory studies or *in vivo* use in laboratory organisms only.
2. Products and Deliverables are supplied for the Customer's personal research activities and noncommercial use. Products and Deliverables must not be sold or otherwise redistributed without KromaTiD's consent.
3. KromaTiD's full Terms of Sale can be found our website.



TECHNICAL SUPPORT:
LIVE CHAT: M-F 9 AM- 5 PM MT
EMAIL: TECHSUPPORT@KROMATID.COM

INQUIRIES AND ORDERS:
EMAIL: SALES@KROMATID.COM
WEB: KROMATID.COM
Ph: 720.815.2898
Fx: 720.815.2902

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
