Protocol and instructions

Karyotype Service

Fixed cells for analysis

Cat K01, and K05

Protocol Version 8.0

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A guide to preparing and shipping fixed cells for karyotype analysis

The results we have seen from labs performing this procedure are quite variable. Be careful to perform the steps as described. We provide a full processing service from live cells. Please consider this as an option.

Whilst we will endeavour to characterise 20 cells (for stable, non-cancerous samples) for poor preparations, finding mitotic figures becomes very time consuming and a full charge will apply if any cells can be analyzed.

Please do not send any samples without contacting us and booking a specific slot.

Preparing cells

This protocol is for preparing cell cultures for karyotype analysis.

Multiple samples can be prepared in parallel using this protocol, however, we recommend fixing no more than 3 x T25 flasks at any one time.

A. Reagent preparation

- 0.05% Trypsin-EDTA (e.g. Thermo Fisher Scientific, cat 25300054)
- Pre-made 0.075 M KCl solution (e.g. Thermo Fisher Scientific, cat 10575090) is recommended

These reagents should be warmed to 37°C prior to use.

- **Freshly prepared** 3:1 Fixative (add 3 volumes of methanol to 1 volume acetic acid). Methanol (e.g. VWR, Cat 20847.307); Acetic Acid (e.g. VWR, cat 20103.330)

Fixative solution should be prepared on the day and chilled in the fridge (4°C) prior to use.

B. Cell requirements

- For each sample, one T25 flask (or equivalent) is required.
- Cells can be prepared with or without a feeder layer.
- For colony culture, colonies should be large enough to be visible by eye.

Culture should be in log phase, undergoing active cell division. Non-log scale cultures will not yield sufficient cells for analysis. Cultures growing as a monolayer should be at least 50% confluent. Media must be changed 16 – 24 hours prior to harvesting to stimulate cell division.

The following procedure is for T25 flasks (25 cm^2 cell growth surface). Any reagent volumes should be adjusted according to the cell growth surface used. For cells grown in suspension, skip steps 4 – 6 (Trypsin-EDTA treatment). Also, if the cells grow in clumps, tap the flask and gently triturate (repeatedly pipette) to produce a single cell suspension.

C. Procedure

1. Adjust media volume to 6 ml.
2. Add 60 µl colcemid (10 µg/ml; e.g. Thermo Fisher Scientific, cat 15210040).
   (If needed, a different volume of media may be used. Add 10 µl of colcemid for every ml of media).
3. Incubate at 37°C for 30 minutes (ESC cultures) or 60 minutes (iPSC cultures).
   (Please contact us for colcemid incubation times for other cell types).
4. Transfer treated media from flask to a 15 ml centrifuge tube containing 500 µl FBS.
5. Add 2 ml pre-warmed Trypsin-EDTA to the flask. Rock gently for 5 – 10 seconds. Then transfer Trypsin-EDTA to the FBS containing centrifuge tube.
6. Add a further 2 ml Trypsin-EDTA to the flask and incubate for 8 – 10 minutes at 37°C. Monitor cell detachment. Tap the flask and gently triturate (repeatedly pipette) to produce a single cell suspension. If clusters do not break down to single cells, add an additional 1 ml Trypsin-EDTA and incubate for an additional 2 – 3 minutes. Some clumps may persist. Where feeder layers are used, these may remain as a stringy mass. This is acceptable and will not affect final analysis.
7. Transfer cells to the centrifuge tube (containing FBS plus Trypsin-EDTA) prepared in steps 4 and 5 above.
8. Centrifuge the tube at 800 – 1000 rpm (approx. 150 x *g*) for 8 minutes.
9. Discard the supernatant and flick tube 20 times to dislodge the cell pellet.
10. Using a dropper or a 2 ml serological pipette add 2 ml pre-warmed 0.075 M KCl drop-by-drop (1 drop/second), gently mix. Adjust total volume to 4 ml with 0.075 M KCl in the same manner as before and gently mix again.
11. Incubate at 37°C for 25 minutes.
    Note: After hypotonic treatment, cells are more fragile. Handle carefully.
12. Using a dropper or a 2 ml serological pipette, **very slowly** (1 drop/second), add 10 drops of cold fixative (3:1 methanol/acetic acid). Tilt the centrifuge tube and make sure the 10 drops of fixative slide down the side of the tube into the mixture. Mix by gently inverting once or twice.
13. Incubate at room temperature for 10 minutes.
14. Centrifuge the tube at 800 – 1000 rpm (approx. 150 x *g*) for 8 minutes.
15. Discard the supernatant and flick tube 20 times to dislodge the cell pellet.

16. **Very slowly** (1 drop/second) with gentle mixing between each 3 drops**,** add 2 ml of cold fixative. Invert to mix. Adjust the volume of fixative drop by drop (1 drop/second) with gentle mixing to 4 ml. Again, invert to mix.
17. Incubate sample at room temperature for 30 minutes.
18. Centrifuge the tube at 800 – 1000 rpm (approx. 150 x *g*) for 8 minutes.
19. Discard the supernatant and flick tube 20 times to dislodge the cell pellet.
20. Add 1.5 ml fixative. Swirl to mix.
21. Transfer the cell suspension to a 2 ml screw cap microcentrifuge tube and seal with parafilm.
    Please do not use Cryovials as these cause the liquid to splash upon opening and can lead to sample loss.

Sample details

A. Requisition form

Please complete a requisition form for each batch of samples being sent to Cell Guidance Systems in advance. The requisition form should be filled and submitted here.

Please ensure that sample vials are labelled correctly, with information matching the requisition form.

Shipping instructions

A. Shipping hazardous materials

The cell fixative used to prepare cells for shipping is a 3:1 ratio of methanol:acetic acid. Both solutions are considered hazardous materials. Cells suspended in this fixative are non-viable/non-infectious and can be shipped by air in limited (excepted) quantities.

Packaging must be in compliance with the harmonized Excepted Quantity provision of the International Transport Association (IATA), International Civil Aviation Organization (ICAO), and the U.S. Department of Transportation (DOT) regulations.

Quantity limits

Cell pellets can be shipped under the Excepted Quantity provision of IATA in no more than 2 ml of fixative per tube (inner packaging unit) and no more than 50 ml of combined fixative volume per box (outer packing unit).
For example, one shipping box can accommodate a maximum of 25 cell pellets (each cell pellet re-suspended in no more than 1.5 – 2.0 ml of fixative).

Dangerous Goods	UN Identifier	Hazard Class	Packaging Group	Excepted amount per inner package	Excepted amount per outer package
Methanol (flammable)	UN1230	3	2	2 ml	50 ml
Glacial acetic acid (corrosive)	UN2789	8	2	2 ml	50 ml

B. Prepare the documents

The instructions provided below are for FedEx, but you can select your preferred courier provider.

1. Complete a FedEx Expanded Service International Air Waybill. Contact your local FedEx office to assist you in completing this form.

Section	Section Heading	Information to Provide
3	Shipment Information	- Non-viable cells in methanol & acetic acid solution - For medical research use only - Dangerous Goods in Excepted Quantity - IATA 2.7
3	Harmonized Tariff Code	Not applicable
4a	Express Packaging Service	Select: FedEx International Priority
6	Special Handling	Select: Yes, Shipper's Declaration not required

2. Prepare a Commercial Invoice and make 4 copies.
   http://images.fedex.com/downloads/shared/shipdocuments/blankforms/commericalinvoice.pdf

Section Heading	Information to Provide
Full Description of Goods	- Non-viable cells in methanol & acetic acid solution, net 2.0 ml - For medical research use only - Non-infectious, non-hazardous, non-toxic - Dangerous goods in Excepted Quantity - IATA 2.7
Harmonized Code	Not Required
Declaration Code	Not Required

3. E-mail the tracking number and shipment delivery date to Cell Guidance Systems at info@cellgs.com.

C. Ship the package

Packaging for cell pellets fixed in methanol/acetic acid must be constructed with 3 layers:

1. **Inner (primary) packaging**. Re-suspend the cell pellet in 1.5 ml of fixative and place it in a labeled 2 ml microcentrifuge tube. Tighten the cap and seal with parafilm. Do not fill the tube to the top. Leave room for liquid expansion.

2. **Intermediate (secondary) packaging**. Wrap the microcentrifuge tube in absorbent paper toweling and place the tube in a larger 50 ml centrifuge tube. Tighten the cap and seal with parafilm.

3. **Outer packaging**. Place the 50 ml tube in a sturdy, well-padded, cardboard box. Do not send samples in envelopes or similar unsuitable packaging. The dimension of the outer box must be at least 100 mm on two of the three sides.

4. Place the following documents in the shipping box and seal it with packing tape.

   - 1 copy of the Commercial Invoice (EU customers do not require this)

- MSDS Sheet for glacial acetic acid
  https://uk.vwr.com/assetsvc/asset/en_GB/id/7669226/contents

- MSDS Sheet for methanol
  https://uk.vwr.com/assetsvc/asset/en_GB/id/7668343/contents

5. Place 3 copies of the Commercial Invoice on the outside of the box, in the plastic sleeve, under the FedEx Expanded Service International Air Waybill

6. Contact your courier to pick up the box, or arrange to deliver the package to a FedEx drop off point.

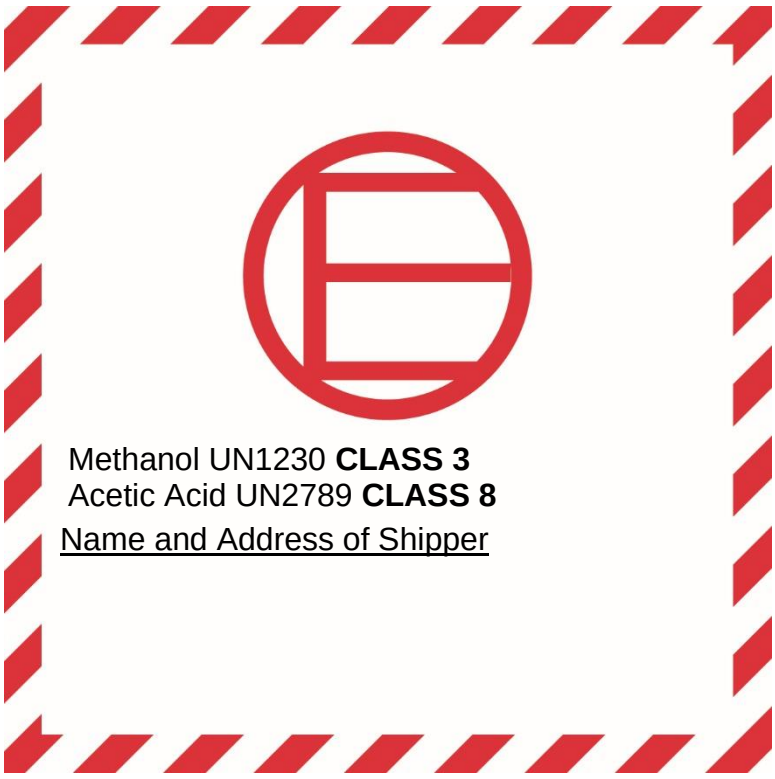
7. Email the tracking number and shipment delivery date to Cell Guidance Systems.

D. Packaging Marks

1. Print an Excepted Quantity Package Mark from the attachment and attach it to the outside of the package.

2. Write the name of the sender in the area designated.

Excepted Quantity Package Mark
From IATA DG Regulations, 50th Ed., Figure 2.7B



Minimum dimensions of this label are: 100 x 100 mm.

CUT OUT AND ATTACH TO EXTERIOR OF PARCEL.

Hatched image can be in RED or BLACK

Below the hatched E marking record:

Name and address of shipper

DISCLAIMER: These materials are provided as a courtesy, to be used as guidelines to assist properly trained shippers. Cell Guidance Systems is not responsible for correct shipping.

E. Delivery address

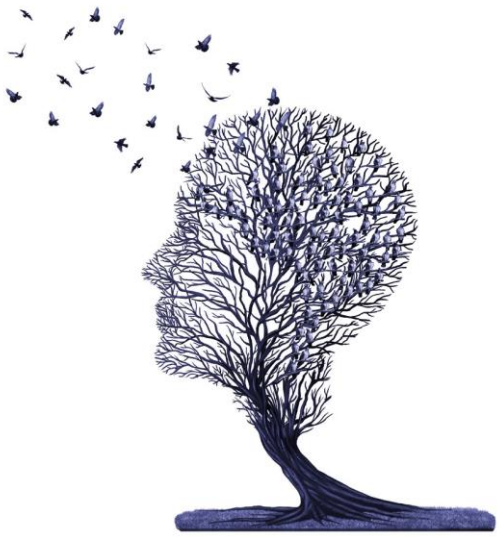
For USA

Attention: Umesh Patel
Cell Guidance Systems LLC
Helix Center
1100 Corporate Square Drive
St. Louis
MO 63132
USA
Tel 760 450 4304

For Europe and Rest of the World

Attention: Michael Jones
Cell Guidance Systems
Maia Building
Babraham Bioscience Campus
Cambridge
CB22 3AT
United Kingdom
Tel +44 (0) 1223 497 115

Cell Guidance Systems' reagents and services enable control, manipulation and monitoring of the cell, both *in vitro* and *in vivo*.



Growth Factors
- Recombinant
- Sustained Release

Exosomes
- Purification
- Detection
- Tracking
- NTA Service

Small Molecules

Cell Counting Reagent

Matrix Proteins

Cell Culture Media
- Pluripotent Stem Cells
- Photostable
- *In Vitro* Blastocyst Culture
- ETS-embryo Culture
- Custom Manufacturing Service

Gene Knock-Up System

Cytogenetics Analysis

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