A guide to preparing and shipping fixed cells for karyotype analysis

Contents

Part I Preparing Cells

Part II Requisition Form

Part III Shipping Instructions

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. Please consider this as an option. For samples we receive as fixed cells, if no cells can be analyzed, there is no charge. However, whilst we will endeavour to characterize 20 cells (for stable, non-cancerous samples) for poor preparations, finding mitotic figures becomes very time consuming and a full charge will be made if any cells can be analyzed.

Part I 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 3x T25 flasks at any one time.

Reagent Preparation

1. 0.05% Trypsin-EDTA (e.g. Thermo Fisher Scientific, Cat#: 25300054)
2. 0.075 M KCl. Pre-made KCl solution (e.g. Thermo Fisher Scientific, Cat#: 10575090) is recommended
These reagents should be warmed to 37°C prior to use.
3. 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)

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 cm² 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.

Procedure

1. Adjust media volume to 6 ml.
2. Add 60 µl Colcemid (10 µg/ml; e.g. Thermo Fisher Scientific, Cat#: 15210040) and incubate at 37°C for 20-30 minutes (ESC cultures) or 40-60 minutes (iPS cultures). For other cell types this incubation time is sample specific. However, for the majority of cell lines, overnight (16h) incubation is adequate. Alternatively, use any different media volume and adjust the amount of Colcemid added (10 µl for every ml of media).
3. Transfer treated media from flask/dish to a 15 ml centrifuge tube containing 500 µl FBS.
4. Add 2 ml pre-warmed Trypsin-EDTA. Rock gently for 5-10 seconds. Then transfer Trypsin-EDTA to the FBS containing centrifuge tube.
5. Add a further 2 ml Trypsin-EDTA to the flask/dish 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.
6. Transfer cells to the centrifuge tube (containing FBS plus Trypsin-EDTA) prepared in steps 3 and 4 above.
7. Centrifuge the tube at 800-1000 rpm (approx. 150 x g) for 8 minutes.
8. Discard the supernatant and flick tube 20 times to dislodge the cell pellet.
9. 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.
10. Incubate at 37°C for 25 minutes.
   **Note:** After hypotonic treatment, cells are more fragile. Handle carefully.
11. Using a dropper or a 2 ml serological pipette, very slowly (1 drop/second), add 10 drops of the 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. Incubate at room temperature for 10 minutes.
12. Centrifuge the tube at 800-1000 rpm (approx. 150 x g) for 8 minutes.
13. Discard the supernatant and flick tube 20 times to dislodge the cell pellet.
14. Very slowly (1 drop/second) with gentle mixing between each 3 drops, add 2 ml fixative, invert to mix. Adjust the volume of fixative drop by drop (1 drop/second) with gentle mixing to 4 ml, invert to mix. Incubate sample at room temperature for 30 minutes.
15. Centrifuge the tube at 800-1000 rpm (approx. 150 x g) for 8 minutes.
16. Discard the supernatant and flick tube 20 times to dislodge the cell pellet.
17. Add 1.5 ml fixative. Swirl to mix.
18. Repeat steps 15-17 two more times (for a total of 3 centrifugation and re-suspension steps). If the pellet is barely-visible/invisible to the naked eye, 1 centrifugation and re-suspension cycle should be enough.
19. 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 significant sample loss.

Part II 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.

Part III Shipping Instructions

The cell fixative used to prepare cells for shipping is a 3:1 ratio of methanol:glacial 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).

<table>
<thead>
<tr>
<th>Dangerous Goods</th>
<th>UN Identifier</th>
<th>Hazard Class</th>
<th>Packaging Group</th>
<th>Excepted amount per inner package</th>
<th>Excepted amount per outer package</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (flammable)</td>
<td>UN1230</td>
<td>3</td>
<td>2</td>
<td>2 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>Glacial acetic acid     (corrosive)</td>
<td>UN2789</td>
<td>8</td>
<td>2</td>
<td>2 ml</td>
<td>50 ml</td>
</tr>
</tbody>
</table>

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

**Tips:**

<table>
<thead>
<tr>
<th>Section</th>
<th>Section Heading</th>
<th>Information to Provide</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Shipment Information</td>
<td>- Non-viable cells in methanol &amp; acetic acid solution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- For Medical Research Only</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Dangerous Goods in Excepted Quantity IATA 2.7</td>
</tr>
<tr>
<td>3</td>
<td>Harmonized Tariff Code</td>
<td>Not applicable</td>
</tr>
<tr>
<td>4a</td>
<td>Express Packaging Service</td>
<td>Select: FedEx International Priority</td>
</tr>
<tr>
<td>6</td>
<td>Special Handling</td>
<td>Select: Yes, Shipper’s Declaration not required</td>
</tr>
</tbody>
</table>

2. Prepare a Commercial Invoice and make 4 copies.


**Tips:**

<table>
<thead>
<tr>
<th>Section Heading</th>
<th>Information to Provide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Description of Goods</td>
<td>- Non-viable cells in methanol &amp; acetic acid solution</td>
</tr>
<tr>
<td></td>
<td>- For research use only.</td>
</tr>
<tr>
<td></td>
<td>- 2.0 ml</td>
</tr>
<tr>
<td></td>
<td>- non-infectious, non-hazardous, non-toxic</td>
</tr>
<tr>
<td></td>
<td>- Dangerous goods in EXCEPTED QUANTITIES</td>
</tr>
<tr>
<td></td>
<td>- IATA 2.7</td>
</tr>
<tr>
<td>Harmonized Code</td>
<td>Not Required</td>
</tr>
<tr>
<td>Declaration Code</td>
<td>Not Required</td>
</tr>
</tbody>
</table>

3. Complete a Cell Guidance Systems Test Requisition Form listing all cultures you are shipping.

**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
   - MSDS Sheet for methanol
   - Test Requisition Form.

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 FedEx 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.

PACKAGE MARKINGS (See Appendix)

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

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

MAILING 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
  UK
  Tel +44 1223 497115
Appendix A

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.