Instructions

Karyotype Service

Live cells for analysis

Cat K02, K07, K06, and K08

Protocol Version 6.0

Contents

A guide to preparing and shipping live cells for karyotype analysis

Preparing cells

A. Cell requirements

- For each sample, one T25 flask (or equivalent) is required.
- Cells can be prepared with or without feeder layer.
- For colony culture, colonies should be large enough to be visible by eye.
- At fixing, culture should be in log phase, undergoing active cell division. Non-log scale cultures will not yield sufficient cells for analysis.

Please note, that for cells classified as Biosafety Level 2 (BSL2) an extra questionnaire is provided, so a Risk Assessment document can be prepared before the live cells are received in our laboratories.

All live cells will be incubated in a standard incubator at the following conditions: 37°C, 5% CO_2 and atmospheric O_2. If the maintenance of the cells requires different parameters, please provide already fixed cells for analysis.

Please note that live cells will not be processed unless the following sample requirements are met:

- Cells are shipped at room temperature in a T25 flask with a solid cap.
- Shipment flasks are full to the brim with cell culture media. This will be approximately 60 ml for a T25 flask, but volumes will vary depending on brand.
- Enough additional cell culture media is supplied in order to allow for sufficient media changes (20 ml per flask).
- Tissue culture flask filter caps are supplied in order to replace solid caps prior to incubation.
    - Please send filter caps still attached to flasks, in order to maintain sterility.
    - Adjustable solid/vented caps are also accepted.
- Cultures growing as a monolayer should be 70 – 90% confluent at the time of harvesting. Therefore, they should be shipped to arrive at Cell Guidance Systems' laboratories, two

days prior to reaching 100% confluency. Samples should be in log phase at the time of fixing.

- Samples should be in transit for no more than 24 hours, and reach us on a Tuesday or Wednesday at the latest.

B. Handling of cells by Cell Guidance Systems prior to fixing

1. At receipt, most of the medium will be removed except for ~6 ml.
2. The plug seal screw caps will be replaced by ventilated caps (from the additional T25 flask provided).
3. The cells will be incubated at 37°C in the presence of 5% CO_2 for ~4 h.
4. Half of the medium of the cells (~3 ml) will be replaced and the cells will be incubated at 37°C in the presence of 5% CO_2 overnight.

The cells are then fixed for karyotype analysis, once sufficient confluency is reached.

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.

B. Questionnaire for Biosafety Level 2 (BSL2)

For cells classified as BSL2, apart from the requisition form, please answer the following questions and send this form back by email.

1. Questions

a. What species are the cells derived from?

b. What is the type of cells?

c. What is the cell line name?

d. What is the geographic/population source of the cells?

e. Human or animal pathogen known to be present? (If yes: mention ACDP or DEFRA classification)

2. Questions

Is the paperwork confirming screening for human pathogens available? If yes, please provide as pdf attachments to the email.

3. Questions

a. Is there risk of disease to humans from microorganisms, cells etc. including colonization, infection, allergenic or toxic effects etc.?

b. Is there risk of adverse effects resulting from the inability to treat disease or offer effective prophylaxis?

c. Will certain groups of people be at increased risk of infection etc. (including immunocompromised individuals, pregnant women, breastfeeding mothers)

4. Questions

a. If the micro-organism is controlled by DEFRA, do you have a DEFRA license and reference number?
   (i) Yes
   (ii) No
   (iii) Not applicable

b. Disease to animal or plants?
   (i) Yes
   (ii) No

c. Adverse effects resulting from establishment or dissemination of the genetically modified microorganism in the environment?
   (i) Yes
   (ii) No

5. Questions

a. Has consent been obtained from the donor for the use of the cells (where required)?
   (i) Yes
   (ii) No
   (iii) Not applicable

Shipping instructions

A. How to send the flasks with live cells

1. Seal the flasks with parafilm.
2. Enclose the flasks in a polystyrene box to insulate from temperature extremes.
3. Ensure the flasks will not move within the box by providing cushioning with bubble wrap.
4. Remember to enclose purchase order.
5. Ship at ambient temperature (do not include ice packs).

We highly recommend that the cells are only in transit for one day/night.
In our experience, cells that have been shipped over two days do not sufficiently recover.

B. Delivery address

Attention: Michael Jones
Cell Guidance Systems
Maia Building
Babraham Research 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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