A guide to preparing and shipping live cells for karyotype analysis

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Part I Preparing Cells

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.
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, two days prior to reaching 100% confluency.

Please note, that for cells classified as Biosafety Level 2 (BSL2) an extra questionnaire will be provided, so a Risk Assessment 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% CO₂ and atmospheric O₂. If the maintenance of the cells requires different parameters, please provide already fixed cells for analysis.

Client Provides
1. Cells shipped (2 days prior to confluence) at Room Temperature in a T25 flask with a solid cap, full with medium.
2. Ventilated caps to fit the T25 flask. This is needed to replace the solid cap for continued culture.
3. Additional media (10 ml per flask).
4. Please use overnight shipping and ensure the cells reach us on a Tuesday or Wednesday at the latest.

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% CO₂ 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% CO₂ overnight.
5. The following day, if high confluency has been reached, the cells are fixed for karyotype analysis.
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.

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

**Question Set 1**

(i) What species are the cells derived from?

(ii) What is the type of cells?

(iii) What is the cell line name?

(iv) What is the geographic/population source of the cells?

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

**Question Set 2**

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

**Question Set 3**

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

(ii) Is there risk of adverse effects resulting from the inability to treat disease or offer effective prophylaxis?

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

**Question Set 4**

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

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

(iii) Adverse effects resulting from establishment or dissemination of the genetically modified microorganism in the environment?
   a. Yes
   b. No

**Question 5**

(i) Has consent been obtained from the donor for the use of the cells (where required)?
   a. Yes
   b. No
   c. Not Applicable
Part III Shipping Instructions

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.*

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