Immortalized Cells FAQ

I want to make sure these cells express my gene of interest before I decide to buy the cell line. Can you provide a sample so this can be tested?

We do not carry out downstream characterization or gene expression profiling of our cell lines. To facilitate your preliminary experiments we can provide an RNA extraction (0.5ug total RNA) or cell lysate (100ug/100ul provided in 62.5mM Tris-HCl, 2% SDS, 10% Glycerol, 50mM DTT, 0.01% w/v Bromophenol Blue) for any of our immortalized cell lines for a small fee. Please inquire directly for more information. The lead time will be around 2 weeks from the time of placing an order (if the item is in stock).

How often do I need to change the media?

The media should be changed every 2-3 days.

Why do these cells need bio safety level II?

In order to be more cautious, we follow the CDC-NIH recommendations that all mammalian sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products. This information can be found in 'Biosafety in Microbiological and Biomedical Laboratories' (1999). Your institution's Safety Officer or Technical Services will be able to make the call as to whether BioSafety Level I is possible with these cells at your site if required.

Do you sell ECM coated T75 flasks?

Yes we can provide a coating service. Please inquire with quotes@abmgood.com.

What can I coat a larger dish to subculture?

We also offer applied extracellular matrix (collagen type I) in liquid form, for the coating of larger flasks and other required plasticware: http://www.abmgood.com/Applied-Cell-Extracellular-Matrix-G422.html

How long can I store frozen vials for?

Cells that are properly frozen using an effective cryoprotective agent can be stored in liquid nitrogen indefinitely without affecting their recovery.

How are live cells shipped?

Live cells require a 1 week preparation time, and they will be shipped with the culture flask filled to the top with complete media and sealed with parafilm. The filled media will avoid the shearing forces of media swishing around the underfilled containers and also protect the cells from drying if the flasks are inverted during the transport. We will also seed the cells to a certain confluency to avoid metabolic waste build up during transport and plate the cells 1 day prior to shipment. In addition, we thoroughly test our cell lines for functional freeze-thaw recovery.

Should the cap of the flask be changed before starting the cell culturing step?

No, there is no need in sterile biosafety cabinets unless it has contacted any non-sterile condition (e.g. touching the contaminated tip, etc.).

What is the recommended storage temperature?

In general, if you received:

Live cells: acclimatize for 3-4 hrs at at the recommended conditions stated for the cell line under the propagation section, and then change media afterwards.
Frozen cells: Immediately place cells in liquid nitrogen; -180C.

How is cell density crucial for drug selection?

If antibiotic selection is applicable to the target cells, we suggest getting rid of all the background cells so that the cell density is kept lower (even 20-30%). However, once the clones are selected by clonal dilution, we don't need the drug to still be present. If needed, the cell density should be towards the higher end since cells are already selected. Any primary cells still present will be depleted as a result of senescence and the cell population that remains will be resistant to the specific antibiotic.

My cells are not detaching, what method do you recommend to trypsinize the cells?

1. Incubate the coated plate containing trypsin solution at recommended temperature indicated in the propagation section for 3-5 min till the cells round up, monitoring from time to time under microscope.
2. Diluting G422 (1:1) with PBS and coating for lesser time. Sometimes the collagen content in G422 is higher and thus make stronger bonding with cells.
3. You can try reducing the incubation time as well for coating the plate to make a thinner layer.

Why is it important to determine the optimal seeding density?

The seeding density we recommend is for when cells are plated to a new vessel. The optimal seeding density should allow cells to attach to the surface and have room to proliferate.

If you seed too little, cells may not attach well to the surface (for adherent cells). Seeding density is important as many cells (adherent or suspension cells) need to be in close proximity for better growth. Cell-cell interactions allow cells to communicate with each other in response to changes in their microenvironment. This ability to send and receive signals is essential for the survival of the cell. In other cases, if the seeding density is too low, cells may attach but a retardation in cell growth is observed.

If you seed too high, the cells will attach but there is insufficient room for further proliferation and they will stop replicating.