

# Fundamental Cell Culture Techniques

Reference: ECACC Laboratory Handbook 4th Edition

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PLEASE READ THIS BEFORE STARTING YOUR CELL CULTURE WORK

Given below are a few of the essential “do’s and don’ts” of cell culture. Some of these are mandatory, e.g. use of personal protective equipment (PPE). Many of them are common sense and apply to all laboratory areas. However, some of them are specific to tissue culture. Follow these simple rules to ensure healthy and reproducible cell cultures.

## The Do’s of Cell Culture: Cell Culture Best Practices

Use personal protective equipment (PPE), (laboratory coat/gown, gloves and eye protection) at all times. In addition, thermally insulated gloves, full-face visor and splash-proof apron should be worn when handling liquid nitrogen.

Use disposable head caps to cover hair.

Wear dedicated PPE for the tissue culture facility and keep separate from PPE worn in the general laboratory environment. The use of different colored gowns or laboratory coats makes this easier to enforce.

Keep all work surfaces free of clutter.

Correctly label reagents including flasks, medium and ampoules with contents and date of preparation.

Only handle one cell line at a time. This common-sense point will reduce the possibility of cross contamination by mislabeling etc. It will also reduce the spread of bacteria and mycoplasma by the generation of aerosols across numerous opened media bottles and flasks in the cabinet.

Clean the work surfaces with a suitable disinfectant (e.g. 70% alcohol) between operations.

Maintain separate bottles of media for each cell line in culture.

Examine cultures and media daily for evidence of gross bacterial or fungal contamination. This includes medium that has been purchased commercially.

Check quality control information for all media and reagents and ideally test performance prior to use.

Keep cardboard packaging to a minimum in all cell culture areas.

Ensure that incubators, cabinets, centrifuges and microscopes are cleaned and serviced at regular intervals.

Test cells for the presence of mycoplasma on a regular basis.

## The Don’ts of Cell Culture: Common Mistakes to Avoid

Do not use antibiotics continuously in culture medium as this can lead to the appearance of antibiotic resistant strains and may mask underlying contamination.

Do not allow waste to accumulate particularly within the microbiological safety cabinet or in the incubators.

Do not have too many people in the lab at any one time.

Do not handle cells from unauthenticated sources in the main cell culture suite. Use a cell supplier that authenticates cells for seed stocks. They should be handled in quarantine until quality control checks are complete.

Avoid keeping cell lines continually in culture without returning to frozen stock.

Avoid cell cultures from becoming fully confluent. Always sub-culture at 70-80% confluency or as advised on the cell culture data sheet.

Do not allow media to go out of date. The shelf life is only 4-6 weeks at +4 °C once glutamine and serum are added.

Avoid water baths from becoming dirty by regular cleaning. Do not allow essential equipment to become out of calibration.