



Troubleshooting Guide – Cell Culture

The following guide can be used to troubleshoot any Cell Culture projects.

If you require any additional information, please email us at info@willowfort.co.uk.

<u>Problem</u>	<u>Causes</u>	<u>Possible Actions</u>
Culture Contamination	Non-sterile surfaces, equipment and reagents	<ul style="list-style-type: none"> • Disinfect all surfaces and equipment before starting cell work • Use equipment pre-sterile
	Spillage on flasks necks of bottle	<ul style="list-style-type: none"> • If possible, passage cells and discard all flasks. • Discard of reagent bottles if contaminated and make fresh reagents.
	Exposing flasks/plates to ungloved hands	<ul style="list-style-type: none"> • Always wear gloves and work in sterile conditions
	Dust/spores from incubator	<ul style="list-style-type: none"> • Check filters in incubators for leaks/holes etc.
	Inadequate sterilization procedures	<ul style="list-style-type: none"> • Always disinfect any equipment, surfaces and reagents with 70% IMS
	Talking, coughing and sneezing over flasks/plates	<ul style="list-style-type: none"> • Avoid working with a cold, or wear a mask
	Contaminated water baths	<ul style="list-style-type: none"> • Regular cleaning and disinfection are recommended.
	Contaminated incubators	<ul style="list-style-type: none"> • Check filters in incubators for leaks/holes etc. • Clean incubators at regular intervals
	Wash back from waste beaker	<ul style="list-style-type: none"> • Use a vacuum to discard the waste reagents

<u>Problem</u>	<u>Causes</u>	<u>Possible Actions</u>
Poor Cell Growth	No/few viable cells after thawing	<ul style="list-style-type: none"> • Use pre-warmed media • Make sure cryoprotectant from media • Store cells below -130°C • Freeze cell cultures as slowly as possible. It is recommended at 1-3°C per minute, preventing any formations of ice crystals
	Poor cell attachment after from stock/passaging	<ul style="list-style-type: none"> • Ensure cells and reagents are evenly mixed • Built up static electricity, by increasing room humidity
	Slow cell growth	<ul style="list-style-type: none"> • Obtain new stock, if cells have been passaged too many times • Obtain a new stock, if cells have become too confluent. Do not let cultures to reach 100% confluency.
	Uneven cell growth	<ul style="list-style-type: none"> • Ensure even mixing of reagents and cells • Check temperature variations if identical cultures are growing irregular.

<u>Problem</u>	<u>Causes</u>	<u>Possible Actions</u>
Precipitates in Media Culture	Temperature	<ul style="list-style-type: none"> • Avoid repeating Freeze-thaw cycles.
	Calcium Salts	<ul style="list-style-type: none"> • Use deionized water to re-dissolve Calcium. Do not add any other components until redissolved and before adding other reagents.
	Humidity	<ul style="list-style-type: none"> • Check the humidity in the culture incubator. • Check for any evaporation from flasks and plates
	Contamination	<ul style="list-style-type: none"> • Remove and discard any cells that are contaminated. Also discard any culture reagents • Disinfect any equipment used for cell culture

<u>Problem</u>	<u>Causes</u>	<u>Possible Actions</u>
Poor Cell Attachment	Contamination	•
	Incubator temperature fluctuations	•
	Insufficient cell surface	•
	Inappropriate environmental gas mixture	•
	Incorrect cell culture media	•
	Incorrect environmental factors	•

<u>Problem</u>	<u>Causes</u>	<u>Possible Actions</u>
Cell Clumping	DNA release	DNase I can be added to digest any free DNA causing cells to clump
	Bivalent cations	Add EDTA to the media to remove Ca ²⁺ /Mg ²⁺ .
	Cell density	Follow the supplier's protocol/instructions for more information
	Mycoplasma contamination	All cells affected and other cell lines in cell incubator should be discarded.

Problem

Causes

Possible Actions

**Cell
Death**

Reagents are poor quality/wrong formulation	<ul style="list-style-type: none"> • Discard of old reagents and replace with fresh. • Double check the correct complete media formulation
Cells are too confluent	<ul style="list-style-type: none"> • Passage cells in the log growth phase. • Do not allow cells to grow further than 80%
Microbial Contamination	<ul style="list-style-type: none"> • See <i>Cell Contamination</i> for more information
Incorrect thawing of culture	<ul style="list-style-type: none"> • Cultures should be thawed rapidly • Instructions should be followed according to the protocol • Any cryoprotectant present in media should be removed as soon as possible
Incorrect culture storage	<ul style="list-style-type: none"> • For long term storage, cells should be stored in -130°C
Cells have been passaged too many times	<ul style="list-style-type: none"> • See <i>Poor Cell Attachment</i>
Environmental factors fluctuate	<ul style="list-style-type: none"> • Check temperature and CO₂ gauges.