

Problem	Possible Reasons	What to do?
Low or no attachment after thawing	Use of inappropriate freezing medium	Check
	Damage during freezing process	Check freezing process
	Improper storage of cells	Check cool chain and storage conditions
	Irreversible damage induced during thawing	Transport cells on dry ice; check water bath temperature and thaw cells according to the recommended protocol
	Forgot to add supplements during media preparation or added wrong supplements	Prepare fresh medium
	Incubator: wrong CO <sub>2</sub> concentration, wrong temperature	Check
	Incubator opened too often	Door should not be opened too often during attachment period
	Inappropriate plasticware	Compare TC dishes from different brands
	Lot number change for plasticware	Use old lot number and compare
	Forgot to coat the TC vessels	Use recommended coating matrix
	Lot number change for coating solution	Use old lot number and compare
	Cap not opened by half turn	Check
	Strong bacterial contamination	Start with a fresh vial; thoroughly disinfect the vial with 70% ethanol before opening it; use aseptic techniques when handling the cells
	Cells aren't anchorage-dependent	Grow cells in suspension culture

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