Trouble Shooting Cell Culture

PromoCell

Problem	Possible Reasons	What to do?
Slow growth after subculture	Damage during the thawing process	Check thawing protocol; damage during thawing often becomes apparent after the first subculture only
	Subcultured too early or too frequently	Check confluence; subcultivate at 70-90% density; 1 - max. 2 subcultivations per week
	Cells have been grown to complete confluency	Trypsinize cells at a subconfluent stage
	Cells plated at too low cell number	Use recommened seeding density
	Damage due to over-trypsinization; trypsin concentration too high	Check trypsin concentration; trypsinize cells at room temperature, check detachment under the microscope
	Forgot to inactivate the trypsin	Use medium with 10% FCS or a trypsin inhibitor to stop the trypsin
	Shift from serum-containing to serum-free medium	Check with medium used before, allow adaptation
	Expiry date of medium exceeded; inappropriate storage conditions	Use fresh medium; store according to manufacturer
	Forgot to add supplements during medium preparation	Prepare fresh medium
	Incubator: wrong CO_2 concentration or temperature; door opened too often	Check
	Lot number change for plasticware	Use old lot number and compare
	Lot number change for coating solution or forgot to coat the plates	Use appropriate coating; use old lot number and compare
	Cap of TC-flask not opened by half turn	Check
	Cells have reached their finite life-span and have become senescent	Freshly isolate or thaw cells from stock
	Cells have started to terminally differentiate	Check culture conditions
	Mycoplasma contamination	Detect and eliminate

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