継代培養中に細胞の回収率と生存率を上げるには

いくつかの要因、または要因の組み合わせにより細胞数や生細胞率の低下が生じる可能性があります。細胞の収量または生細胞率に満足できない場合は、以下の情報を利用して、今後の培養の成功率を高めるようにしてください。

細胞の収率の改善

細胞の収率が悪い（50%未満）場合、以下の表を使用して原因と対策を特定してください。その後1つ以上のフラスコで継代培養を行い、適切な解決策を適用します。

状態	考えられる原因	改善策
Majority of cells did not detach	1. Inactive or cold Trypsin/EDTA	1. Use Trypsin/EDTA at room temperature
	2. Improper storage of Trypsin/EDTA	2. Store at -20℃ until ready for use; thaw and allow it to come to room temperature briefly before subculturing
	3. Exposure time to Trypsin/EDTA was too short	3. Exposure time to Trypsin/EDTA is usually 5 – 6 minutes
	4. Trypsin/EDTA has been neutralized	4 .Be sure to rinse the culture completely with HEPES-BSS before trypsinization
	5. Vessel was not rapped firmly	5. Use a moderate amount of force when rapping during trypsinization
Low yield, 95% of the cells detached but the yield was low	Culture was under confluent at trypsinization	Be sure to trypsinize at 60 – 90% confluence with numerous mitotic figures throughout the flask

細胞生存率の改善

生細胞率が低い（50%未満）場合、以下の表を使用して原因と対策を特定してください。その後1つ以上のフラスコで継代培養を行い、適切な解決策を適用します。

低生存率 (<50%)

状態	考えられる原因	改善策
Trypsin/EDTA damaged the cells	1. Used another vendor's Trypsin/EDTA	1. Use only Clonetics™ Trypsin
	2. Exposure time of the cells to Trypsin/EDTA was too long	2. Do not trypsinize longer than 7 minutes
	3. Trypsin/EDTA was used above room temperature. Trypsin becomes more active at temperatures above room temperature	3. Do not use even mildly heated Trypsin/EDTA
	4. Failed to neutralize the trypsin Prolonged exposure to trypsin will damage cells	4. Neutralize the Trypsin/EDTA with Trypsin Neutralizing Solution to eliminate cell damage due to trypsin
	5. Vessel was rapped too firmly during trypsinization. Rapping too hard to release cells causes cell membrane damage	5. Use moderate force when rapping flask to dislodge cells during trypsinization
Culture vessel was too confluent; was completely covered with cells	Culture was too confluent at trypsinization	Be sure to trypsinize at 60 – 90% confluence with about five mitotic figures per field of view
Cell growth slowed before 80% confluence and cells look dull and non-refractile	The most probable cause is failure to increase the volume of medium used as the cell confluency increased The cells become mildly starved and are not able to recover after trypsinization	Change medium and increase volume as recommended. Please observe all guidelines