

Problem	Possible Reasons	What to do?
Cells do not detach or detach very slowly	Cells have been grown to confluency	Trypsinize cells at a subconfluent stage
	Forgot washing step with HepesBSS	Perform washing step to remove residual medium
	Trypsin solution too cold	Use warm trypsin (room temperature or heated to 37°C)
	Trypsin solution too old	Check expiry date; use fresh solution; when stored at 4°C, trypsin has a shelf life of 6 weeks
	Incubation time too short	Use proper incubation time until 80% have detached Please note: some epithelial cell types adhere very firmly and need prolonged incubation times. To avoid irreversible damage by over-trypsinization, the use of accutase is recommended. Accutase is very gentle to the cells, preserves the surface receptors and doesn't affect the cellular viability during extended contact with the cells

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