



## Troubleshooting Guide for Ultra BL21 (DE3) and Ultra BL21 (DE3) pLysS Competent Cells

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
<b>Low transformation efficiencies</b>	There may be DNA impurities present.	Ensure that the DNA does not contain any protein, detergents or ethanol.
	There may be too much DNA.	Transformation efficiencies are calculated using 10pg pUC19. Transformation efficiencies will decrease with increasing amounts of DNA; however, total numbers of clones will increase with the amount of DNA.
	The cells may have been improperly handled or stored.	<ul style="list-style-type: none"> <li>• Cells should be thawed on ice and used immediately. Do not refreeze cells and do not vigorously mix cells by vortexing. Cells must be stored at -70°C to guarantee stability.</li> <li>• Transformation should be done in the tube/well that contains the cells.</li> <li>• Transformation should be done according to the recommended protocol to ensure the guaranteed transformation efficiency.</li> </ul>
	You may be using a large plasmid.	Transformation efficiencies are calculated using pUC19. Larger plasmids will result in lower transformation efficiencies.
	The ligation may have been inefficient.	Double check the cloning strategy, enzymes and concentrations of vector and insert. Set up a control for each step of the cloning procedure.



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<b>Little or no protein expression detected following induction</b>	The vector may have a weak promoter or there may be a lack of good cloning design.	Ensure that the gene of interest is in the correct frame with the fusion tag and stop elements. Sequence the construct to ensure that no undesired changes have been introduced during the cloning steps.
	The concentration of IPTG may be too low.	Increase the concentration of IPTG.
	The protein may be insoluble.	Use denaturing conditions to solubilize the protein
	The protein does not express well in <i>E. coli</i> .	Use a different expression system.
<b>Cell death</b>	The protein you are trying to express may be toxic.	You need tighter control over basal expression; select a different expression host, like BL21 (DE3) pLysS chemically competent cells.
<b>Insoluble protein</b>	Temperature, IPTG concentration and fusion tags can affect solubility.	<ul style="list-style-type: none"> <li>• Lower the temperature during induction.</li> <li>• Lower the IPTG concentration.</li> <li>• Use a different fusion tag.</li> </ul>
<b>No or very few colonies seen</b>	The protein may be toxic and there may be high basal expression.	You need tighter control over basal expression and should select a different expression host, like BL21 (DE3) pLysS chemically competent cells.
	You may have the wrong antibiotic selection.	Ensure that the correct antibiotic selection was used.
	The cells may have lost competency.	Test the efficiency of the cells with the control DNA.



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For additional troubleshooting assistance, please contact Edge BioSystems at:

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