TROUBLESHOOTING GUIDE
Problems and Solutions

Possible causes of problems that could appear during the purification protocol of biomolecules are listed below. The causes described are theoretical and it is always advisable to contact our team with your specific problem.

The table delineates the potential problems at each step in the protocol that might explain poor performance.

1. SAMPLE APPLICATION

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
LOW PROTEIN YIELD	Problems with vector construction	- Ensure that protein and tag are in frame.
	Poor protein expression.	- Optimize bacterial expression conditions.
	Fusion protein forms inclusion bodies.	- Lower the growth temperature from 37ºC to 30 – 15ºC.
	Extraction may be insufficient.	- Check extraction conditions (lysozyme, sonication). - Use up to 2% of a non-ionic detergent to improve cell disruption and/or solubilization of the fusion protein.
HIGHLY DILUTED OR CONCENTRATED SAMPLE	Highly diluted sample.	- It is preferable to concentrate the sample before its purification in the column. - Another solution is to carry out an adsorption step in batch format and pack the column with the resultant resin of the adsorption step.
	Highly concentrated sample.	- It is preferable to make a previous dilution of the sample before its purification in the column.

2. ADSORPTION

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
TARGET PROTEIN DOES NOT BIND EFFICIENTLY	Sonication may have been too severe.	- Choose milder sonication conditions. Over-sonication can alter the conformation of the GST moiety and prevents the fusion protein from binding to Glutathione Agarose.
	Reducing agent missing.	- By adding DTT to the lysis buffer (final concentration 5 mM) prior to cell lysis can significantly increase binding of some fusion proteins.
	Concentration of fusion protein is too dilute.	- Concentrate the sample. If the sample is too dilute, target protein may not bind efficiently. Yield depends on the concentration of the fusion protein in the lysate.
	Flow rate too high.	- Try to use slower flow rates or make the adsorption in batch to allow a better contact between resin and fused protein.

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OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
TARGET PROTEIN DOES NOT BIND EFFICIENTLY	Channels have formed in the column so the sample runs mainly through these undesirable channels.	- Re-pack column.
	Inadequate binding conditions.	- Check the conditions.
	Column capacity exceeded.	- Apply less fused protein to the column.
	The resin has been previously used during several purification cycles. This causes a diminution of the binding capacity. This diminution varies in each case and increases with the number of purification cycles of the resin.	- Clean resin according to the Procedure of use or use freh resin. Note: Immobilized glutathione can be degraded by γ-glutamil transpeptidase activity in E. coli cell lysates.

3. ELUTION

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
POOR PROTEIN PURITY	Sonication may have been too severe	- Choose milder sonication conditions. Over-sonication can lead to the co-purification of host proteins with the GST-fused protein.
	Degradation of GST fusion protein	- Check it. If it has been degraded, make the purification at lower temperatures (4ºC) reducing the degradation. Try to reduce the purification step times. Keep the samples and buffers on ice to reduce the activity of proteases. - Add protease inhibitors. - Use a protease-deficient host. Multiple bands may be the result of partial degradation by host proteases during cell growth.
	Insufficient wasting stage.	- Increase the number of washes with PBS.
	Co-purification of chaperonins	- Several chaperonins, that are involved in protein folding, may co-purify with GST fusion proteins, e.g. DnaK (~70 kDa), DnaJ (~37kDa), GrpE (~40 kDa), GroEL (~57 kDa), GrpE (~40 kDa), GroEL (~57 kDa), GroES (~10kDa). Several additional purification steps have been described. E.g. co-purification of DnaK can be avoided by treating the cell lysate with 5 mM $MgCl_2$ and 5 mM ATP prior to purification. DnaK can be dissociated from other components in the presence of ATP and Mg^{2+}.

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OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
TARGET PROTEIN ELUTES POORLY	Too smoth elution (low elution volume)	- Increase the volume of elution Buffer. Depending on the nature of the fusion protein and the amount of protein loaded, additional elution steps or buffer volume is required.
	Flow rate too high.	- Decrease flow rate during elution.
	Inadequate elution conditions	- Check the buffer and pH of the elution buffer. Note: In some cases increase to 50 mM reduced glutathione improves the elution.

WARNINGS TO THE USER

Activated Agarose Beads are for laboratory use only. Not for use in diagnostic or therapeutic procedures.

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