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| **Step** | **Problem** | **Possible Reasons** | **Solution** |
| 3 | Unstable ITC baseline or baseline shift | Sample cell and syringe are dirty | Thoroughly wash the cell and syringe |
|  |  | Air bubbles in the sample cell | Remove air bubbles. Take care not to introduce bubbles when filling the cell and syringe |
| 13 | Absence of a PCR product after fusion PCR | Fusion of gene did not take place | Recheck the primers and repeat the reaction by slightly varying the annealing temperature |
|  | Multiple PCR products after fusion PCR | The primers may have bound to related sequences elsewhere in the template | Redesign the primers and repeat the reaction |
| 17 | No protein in elution | Cell culture conditions are not suitable | Try different cell culture conditions by varying the IPTG concentration and temperature for induction and induction period |
|  |  | Protein is in the insoluble fraction | Use a refolding method or use a solubility tag to make the protein soluble |
|  |  | No binding with Ni-NTA resin | Use fresh Ni-NTA resin and equilibrate with lysis buffer for a minimum of 1 h |
|  |  | Protein eluted away during wash steps | Remove imidazole from wash buffers and repeat the assay |
| 18 | No protein peak observed in the elution profile | Protein has leaked due to tubing connections. | Check the connections of the loop. Try to reconnect and inject the protein |
|  |  | UV lamp is not sensitive; this is particularly a concern if the protein concentration is low | Change the UV lamp for better sensitivity |
| 18 | Protein eluted in void volume | Protein shows higher order oligomerization | Optimize buffer conditions to prevent aggregation |
|  |  | Binding partners may not be interacting to form an intact complex | Optimize the length of the linker to retain natural interactions between the binding partners |