Preparing Total Cell Protein

Introduction

This is the easiest way to determine if your protein is being expressed. Once you know this, you can go on to determine whether if is in the soluble or insoluble fractions of the cells. (See How to Determine if Your Protein is Soluble or Insoluble

1. Grow induced cells to OD 600 0.6-3
2. Collect cells by centrifugation of 1.5mL of culture.
3. Resuspend cells in 1/10 volume of (150 lambda) 10 mM Tris-Cl pH 8.
4. Remove a 10 uL aliquot and add to an equal volume of SDS-PAGE buffer.
5. Heat to 75 degrees C for 10 minutes and wad onto gel.

NOTES:

- make sure to include a negative control (eg: vector alone without your gene fused to the carrier *or* cells alone without any vector transformation *or* both)
- if your carrier is Protein A or GST then do a Western Blot and probe with either Rabbit IgG-AP (PrA) or with Rabbit anti-GST antibody (primary) then Goat and Rabbit IgG-AP (secondary) (GST); visualize your fusion proteins.