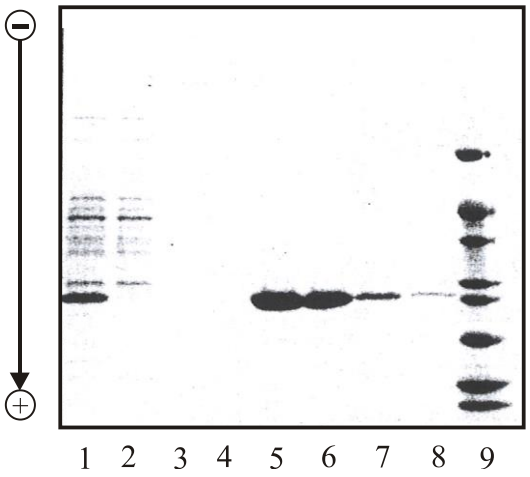
PURIFICATION OF A HIS-TAGGED PROTEIN

Terms to be familiar with before you start to analyze the figure

*cDNA cloning * expression vector * affinity chromatography * washing of colum * elution of column * SDS-polyacrylamide gel electrophoresis * Coomassie blue staining*

The figure



Using recombinant DNA technology, specific proteins can be labelled with a **histidine tag**. The HIS-tagged protein can then be expressed in host cells and purified from cell extracts taking advantage of the ability of nickel-coated agarose beads to bind the HIS-tagged protein selectively.

The figure shows the SDS polyacrylamide gel electrophoresis of fractions of an affinity chromatography performed on a nickel/agarose column. The gel was stained with Coomassie blue after electrophoresis.

Sample 1:	whole cell extract to be loaded onto the column;
Sample 2 to 4:	the extract was loaded and the column was washed with sample buffer;
Sample 5 to 8:	elution of the column;
Sample 9:	molecular weight marker.

Study the protein-stained gel and interpret the results.

1. Interpret the difference between sample 1 and 2 !
2. Interpret the difference between sample 2 and 4 !
3. Interpret the difference between sample 4 and 5 !
4. Interpret the difference between sample 1 and 5 !

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The source of the figure

SIGMA Protein Expression catalog, 2002/2003, p39.

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