# assays: protein

## The Interfering Substance…a Protein Assay Fact of Life

Ideally, an assay should be compatible with all substances commonly present in protein samples. Each protein assay method, however, exhibits its own particular sensitivity to the presence of commonly encountered biochemical reagents in the protein sample. Various techniques in the laboratory require protein samples to be dissolved in buffers containing detergents, chaotropic agents and antimicrobial preservatives. Some of these additives can affect the results of an assay. These compounds then become known as interfering substances, which can affect the assay in one of the following ways:

- They can suppress the color response of a protein
- They can artificially enhance the response of a protein or cause elevated background

The interference from many common substances can be compensated for in the blank designed for a specific assay. To avoid significant interference, the standard curve must be made up in the same diluent that is used for the unknown protein. When only a rough estimate of protein concentration is needed, a "blank only" correction can be used. In this case, a blank is made up in the diluent of the unknown protein to correct for its raw absorbance. The concentration of the unknown protein is then determined from a standard curve that is obtained from a standard protein dissolved in water or saline.

Often interfering substances can overwhelm the assay, making the assay impossible to perform. In these situations, the interfering substance is removed from the protein by precipitation with trichloroacetic acid or acetone before the assay is performed. Gel filtration chromatography performed prior to sample assay can also be used to remove these materials.

When developing a protein assay it is considered essential to identify those substances that are compatible with or may interfere with the assay. Characterizing the assay in this manner helps to further define the utility of the assay.