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a Perbio Science Company

3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

Protein stability and storage

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Introduction

Proteins comprise an extremely heterogeneous class of biological macromolecules. They are often unstable when not in their native environments, which can vary considerably among cell compartments and extracellular fluids. Each protein may have specific requirements once it is extracted from its normal biological mileu. If these requirements are not satisfied, the protein can rapidly lose its ability to perform specific functions. Proteins can lose activity as a result of proteolysis, aggregation and less than optimal storage conditions.

There is often a need to store purified proteins to retain the original structural integrity and/or activity of the protein for an extended period of time. This 'shelf life' can vary from a few days to more than a year and is dependent on the nature of the protein and the storage conditions used. Optimal conditions for storage are distinctive to each protein; however, it is possible to suggest a few general guidelines.

Common conditions for protein storage are summarized and compared in Table 1. Generally, there is a tradeoff associated with each method. Proteins stored in solution at 4°C can be dispensed conveniently as needed but require more diligence to prevent microbial or proteolytic degradation; such proteins may not be stable for more than a few days or weeks. By contrast, lyophilization allows for long-term storage of protein with very little threat of degradation, but the protein must be reconstituted before use and may be damaged by the lyophilization process.

Table 1. Comparison of Protein Storage Conditions

	Storage Condition			
Characteristic	Solution at 4°C	Solution in 25-50% glycerol or ethylene glycol at -20°C	Frozen at -20° to -80°C or in liquid nitrogen	Lyophilized (usually also frozen)
Typical shelf life	1 month	1 year	Years	Years
Requires sterile conditions or addition of antibacterial agent	Yes	Usually	No	No
Number of times a sample may be removed for use	Many times	Many times	Once; repeated freeze-thaw cycles generally degrade proteins	Once; it is impractical to lyophilize a sample multiple times

General Considerations for Protein Storage

Temperature:

Generally, proteins should be stored at ≤4°C in clean, autoclaved glassware or polypropylene tubes. Storage at room temperature often leads to protein degradation and/or inactivity, commonly as a result of microbial growth. For short term storage of 1 day to a few weeks, many proteins may be stored at 4°C.

For long term storage from 1 month to 1 year, some researchers choose to bead single-use aliquots of the protein in liquid nitrogen and store it in clean plastic containers under liquid nitrogen. This method involves adding the protein solution drop-wise (about 100 µl each) into a pool of liquid nitrogen, then collecting the drop-sized frozen beads and storing them in cryovials under liquid nitrogen.

Freezing at -20°C or -80°C is the more common form of frozen protein storage. Avoid repeated freeze-thaw cycles, which decrease protein stability. Instead, prepare small working aliquots so that, once thawed, the protein solution will not have to be refrozen. Adding 50% glycerol or ethylene glycol (see Additives section below) will prevent solutions from freezing at -20°C, enabling repeated use from a single stock.

Protein Concentration:

Dilute protein solutions of less than 1 mg per ml are more prone to inactivation and loss as a result of low-level binding to the storage vessel. Therefore, it is best to store proteins in more concentrated form. However, the addition of a carrier protein, such as purified BSA (final concentration of 10-15 mg/ml), to dilute protein solutions helps to protect against such degradation and loss.

Additives:

Many compounds may be added to protein solutions to lengthen shelf life:

- Cryoprotectants such as glycerol or ethylene glycol to a final concentration of 25-50% help to stabilize proteins by preventing the formation of ice crystals at -20°C that destroy protein structure.
- Protease inhibitors prevent proteolytic cleavage of proteins (Table 2).
- Anti-microbial agents such as sodium azide (NaN_3) at a final concentration of 0.02-0.05% (w/v) or thimerosal at a final concentration of 0.01 % (w/v) inhibit microbial growth.
- Metal chelators such as EDTA at a final concentration of 1-5 mM avoid metal-induced oxidation of –SH groups and helps to maintain the protein in a reduced state.
- Reducing agents such a dithiothreitol (DTT) and 2-mercaptoethanol (2-ME) at final concentrations of 1-5 mM also help to maintain the protein in the reduced state by preventing oxidation of cysteines.

Table 2. Common Protease Inhibitors

Protease Inhibitor	Target Protease	Working Concentration
PMSF (Phenylmethylsulfonyl fluoride)	Serine proteases	0.1 – 1 mM
Benzamidine	Serine proteases	1 mM
Pepstatin A	Acid proteases	1 µg/ml
Leupeptin	Thiol proteases	1 µg/ml
Aprotinin	Serine proteases	5 µg/ml
Antipain	Thiol proteases	1 µg/ml
EDTA and EGTA	Metalloproteases	0.1 – 1 mM

Storage Conditions for Antibodies and Antibody-Enzyme Conjugates

Antibody stock solutions (e.g., 1 mg/ml) often may be stored at 4°C for days to weeks without significant loss in activity. For increased stability, glycerol or ethylene glycol may be added to a final concentration of 50% and the antibody stored at -20°C. Alternatively, the antibody solution may be stored in small working aliquots at -20°C to avoid repeated freeze-thaw cycles. Anti-microbial agents such as sodium azide or thimerosal may be added to avoid microbial growth.

Generally, antibody conjugates are best stored at -20°C with glycerol or ethylene glycol added at a final concentration of 50%. Although some enzyme conjugates may be stored at -20°C without cryoprotectant, frozen stocks must be as single use aliquots to prevent repeated freeze-thaw cycles; alkaline phosphatase conjugates are particularly sensitive to freezing. Conjugates typically maintain good activity for 1-2 years if stored at -20°C with glycerol or ethylene glycol. However, contaminants in cryoprotectants may affect enzyme activity, and few researchers take steps to ensure the purity of the cryoprotectant used. Pierce offers Ethylene Glycol (Product No. 29810) that is suitable for enzyme storage because impurities have been removed during the manufacturing process. Ethylene glycol does not support microbial growth, making it preferable to glycerol.

Pierce also offers SuperFreeze™ (Product No. 31503) and Guardian™ (Product No. 37548) Products, which are multi-component cryoprotectants that provide buffered anti-freeze conditions for the storage of horseradish peroxidase (HRP) conjugates. SuperFreeze™ Peroxidase Conjugate Stabilizer allows freezer storage of peroxidase conjugates, substituting for glycerol or ethylene glycol and ensuring stable liquid storage at -20°C. Guardian™ Peroxidase Conjugate Stabilizer/Diluent allows room temperature or 4°C storage of peroxidase conjugates in diluted form (as low as 10 ng/ml). Working dilutions for ELISA or Western blotting be prepared and stored up to 18 months at 4°C.

Related Pierce Products

29810	**Ethylene Glycol (50% aqueous solution)**, 200 ml
31503	**SuperFreeze™ Peroxidase Conjugate Stabilizer,** 25 ml
37548	**Guardian Peroxidase Conjugate Stabilizer/Diluent,** 200 ml
37552	**Guardian Peroxidase Conjugate Stabilizer/Diluent,** 1 liter
78410	**Halt™ Protease Inhibitor Cocktail kit with EDTA,** 2 ml
78415	**Halt™ Protease Inhibitor Cocktail EDTA free,** 1 ml
36978	**PMSF (Phenylmethylsulfonyl fluoride),** 5 mg

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