# GLYCEROL IN THE LYOPHILISATION PROCESS:

## UNDERSTANDING ITS EFFECTS

BIOFORTUNA

## WHAT IS GLYCEROL?

Glycerol, also known as glycerin, is a colorless, odorless, and sweet-tasting viscous liquid that is hygroscopic and soluble in water. It is a simple polyol compound, consisting of three carbon atoms, each attached to a hydroxyl group (–OH). Glycerol is a byproduct of the soap-making process and the biodiesel production process, and it is widely used in food, pharmaceuticals, and cosmetics due to its moisturising properties and ability to act as a solvent and preservative.

Additionally, glycerol plays a crucial role in biological systems as a component of triglycerides and phospholipids, essential for energy storage and cellular structure.

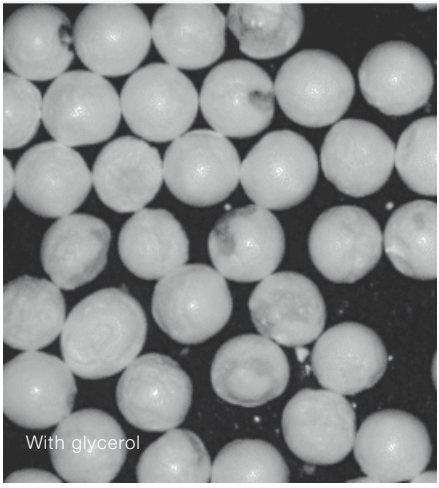
## WHY DO REAGENTS CONTAIN GLYCEROL?

Many commercially available diagnostic reagents, particularly temperature sensitive proteins such as enzymes and antibodies require freezing for long term storage. These are often supplied in a storage buffer system containing glycerol at concentrations between 10 and 50%w/v. Glycerol is added to the storage buffer as a cryo-preservative to prevent the stresses of freezing and thawing from denaturing the protein.

### What impact does glycerol have on lyophilised formulations?

During lyophilisation the glycerol concentration in a formulation increases as water is removed by sublimation during primary drying. This has a plasticising (softening) effect on the lyophilised structure which then becomes glassy during secondary drying. The result is a product that may not be completely dry or will have poor structural appearance. This in turn will impact the product's stability and reconstitution properties.

The carryover of residual glycerol from reagents into the final formulation can vary in concentration significantly. Whilst some lyophilised formulations can tolerate relatively low concentrations of glycerol, we typically see impacts on drying and appearance above 0.1%w/v and sometimes significantly lower depending on the formulation.


With glycerol


glycerol-free

## WHAT SHOULD I DO IF I SUSPECT MY REAGENTS CONTAIN GLYCEROL?

Many of our Customers initially approach us with little or no understanding of the lyophilisation process, having already invested a significant amount of resource developing an optimised ‘wet’ assay formulation only to discover it may not be compatible for lyophilisation due to glycerol and other non-compatible components.

Consider the final intended formulation and its manufacturing process at the earliest point in your design and development process. We can help you co-develop your critical chemistries by offering advice or conducting small feasibility trials on your behalf.

## ARE THERE COMMERCIALLY AVAILABLE REAGENTS THAT DO NOT CONTAIN GLYCEROL?

Several suppliers of critical reagents for use in diagnostics recognise the impact of glycerol on lyophilisation and provide options that are either glycerol-free or ‘lyo-compatible’. Lyo-compatible reagents contain either reduced levels of glycerol or increased concentration of the stock protein so the glycerol becomes diluted to a negligible amount in the final formulation.

There are some formulated reagents such as PCR master-mixes that may be classed as ‘lyo-ready’ meaning they contain all necessary components for lyophilisation at recommended lyophilisation parameters.

These options can be convenient for use but are not always compatible in certain assay formats and can also have a significant cost implication.

## IS IT POSSIBLE TO REMOVE GLYCEROL FROM A REAGENT?

The simple answer is yes but there can be some challenges associated with this strategy as other components may also be co-removed which will impact performance.

We use a process referred to as ‘buffer exchange’ which essentially swaps the original buffer containing glycerol that the critical protein/molecule is supplied in for a glycerol free buffer.

There are three methods of buffer exchange based on size exclusion that our trained biochemists employ depending on the application: Dialysis, Diafiltration and Gel filtration.

**Dialysis** works by diffusion through a semi-permeable membrane with a known molecular-weight cutoff (MWCO) due to the size of its pores. The protein sample containing the glycerol and an exchange buffer solution are placed on opposite sides of the membrane in the form of a cartridge or tubing. The protein (which should be much larger than the membrane pores) is retained on the sample side of the membrane whilst the smaller glycerol molecules diffuse freely through the membrane and approach an equilibrium concentration with the buffer exchange solution (dialysate). The volume ratio of dialysate to sample should be greater than 200:1 to ensure that the glycerol is reduced to a negligible level.

**Diafiltration** works in a similar way to dialysis as it also uses a semi-permeable membrane to separate large proteins from low molecular-weight molecules. However, unlike dialysis, which relies on passive diffusion, diafiltration involves forcing the original buffer solution through the membrane by positive pressure or centrifugation force. The target protein becomes concentrated to a smaller volume as the buffer containing the glycerol is forced across the membrane as the eluate. The volume of eluate is replaced by a fresh glycerol-free buffer. When this is repeated several times the glycerol is reduced to a negligible level.

**Gel filtration** buffer exchange is performed by first equilibrating the column containing a porous resin of known pore size with a glycerol-free buffer. The protein buffer containing the glycerol is then passed through the column. The protein is too large to enter the pores and will quickly pass through the column. In contrast, buffer salts and glycerol will enter the pores of the resin, slowing their rate of migration through the resin bed. This reduction in flow rate causes the larger protein to become separated from the slower, smaller molecules and collected into the new buffer formulation.

# GET EXPERT TECHNICAL ADVICE

Biofortuna is a specialist contract development and manufacturing organisation providing genomic services, custom development and manufacturing support to the *in vitro* diagnostic, consumer health, life sciences and pharma biotech sectors.

As an FDA registered, ISO 13485 and ISO 17025 accredited provider, we integrate our quality management system and design control standards within all our assay design, development and manufacturing projects.

Contact us today for an informal discussion around how we can help you bring your assays to market faster.

E&OE excepted: 12/24


Winner of the Technical Service Award for our Lyo bead development and manufacturing service

BIOFORTUNA

+44 151 334 0182

www.biofortuna.com

Biofortuna Limited
2 Tenth Avenue
Deeside Industrial Park
Deeside. CH5 2UA

MEMBER BIVDA 2024

UKAS TESTING
7816

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED
MD 731514