



General Technical Information – Creative PEGWorks® Hyaluronic Acid (HA) Products

Molecular Weight: The value of polydispersity (PDI) of hyaluronic acid ranges from 1.5-2.0. The MW was confirmed by GPC with polystyrene as calibration standards.

Solubility: Polysaccharide reagents are generally soluble in water including DI water and buffer. Hyaluronic acid due to the presence of acid groups, buffered solution at pH >6 to neutral or slightly basic significantly enhance its solubility. When the carboxylic groups exist as salt of Na, K etc, the solubility in DI water is higher. HA is also soluble in DMSO which is a useful co-solvent along with water to conduct chemistry reactions that are sensitive to water or susceptible to hydrolysis. Solubility goes down as the MW increases. Rehydration takes time up to overnight and sonication might help, particularly for high MW HA. The solution becomes more viscous as the concentration increases.

Sources: The source of our hyaluronic acid is from microbial fermentation with *Streptococcus zooepidemicus*. Our HA products are free of bovine spongiform encephalopathy (BSE).

Physical Form: Polysaccharide products generally appear as white or off-white powder, unless it is dye-labeled. The final product powder is obtained through lyophilization.

Storage Condition: Polysaccharide products shall be stored in the original form as received in a freezer at -20°C or lower for long term storage. Stock solutions that DO NOT contain oxygen or moisture sensitive functional groups (e.g. NHS, thiol etc) may be temporarily stored in a refrigerator or ambient temperature for multiple days. Stock solution should avoid repeated freeze-and-thaw cycles. Also, light and oxygen sensitive products including thiols and those with unsaturated carbon-carbon double bonds such as maleimide, DBCO, acrylate and dyes, ideally shall be stored away from light (i.e. wrapped with aluminum foil) and in an air-free atmosphere. The best way to achieve inert atmosphere is to purge the vial with nitrogen or argon in an inert gas-filled glove box. To prepare stock solution of oxygen sensitive reagents, degassing the solvent with nitrogen or argon is preferred. Light sensitive products are bottled in amber glass vials or plastic vials. Please contact us if you concern the storage and handling of a particular reagent.

Handling: Polysaccharides are highly hygroscopic and absorb moisture from air quickly. Follow these steps to aliquot: 1) Allow vials to thaw and equilibrate to room temperature before opening the vial; 2) Open vials and weigh the quantity you need quickly; 3) Flush vials with dry argon or nitrogen (if you have access to nitrogen or argon). Caution: adjust gas flow so that dry powders will not be blown away; 4) Tightly cap vials and wrap the cap/neck with parafilm; 5) Store in a freezer at or below -20°C.

Shelf-life: When stored as recommended, polysaccharide products typically have 3 years of shelf life. Please refer to COA for the manufacture date and expiration date.

Degree of Substitution (DoS) or Labeling Degree: DoS (%) is defined as the percentage of monomers of each polysaccharide chain is functionalized or labeled with a particular functional group or dye. For example, HA-FITC, DoS 5%, MW 200k: 5% means that 5 mol% (that is, one out of twenty) of monomer units of the HA polymer is fluorescently labeled with FITC dyes. HA has a molecular formula of $(C_{14}H_{21}NO_{11})_n$, where n is the number of repeating disaccharide monomer units. The MW of each disaccharide monomer is 379. The number of monomer units in HA MW 200k is 528 (MW 200,000 divided by the molecular mass of one monomer unit 379); the number of monomers that are labeled with FITC dye is approximately 26 (5% * 528).