Molecular Biology Chemicals

For Molecular Biology research experiments highest quality chemicals are a prerequisite to ensure exact and reproducible results as traces of chemical impurities can interfere in the results or during the analysis. HiMedia provides a broad range of ultrapure, high quality Molecular Biology grade chemicals which can be used for all fundamental experimental methods in the field of Molecular Biology. MB grade chemicals are initially evaluated for the following aspects :

Physical parameters : All chemicals are initially tested for physical parameters as per HiMedia's highest quality specifications.

Chemical Parameters : Each and every Molecular Biology grade chemical is tested for chemical parameters at the ultra modern testing facility. They are evaluated for various molecular biology applications.

Nuclease Testing : Chemicals are tested for the presence of enzyme impurities such as DNases, RNases, and proteases as these chemicals may be used for various Molecular Biology applications.

Functionality Test : All chemicals are functionally evaluated i.e. performing a protocol with that chemical as the variable in an assay where the chemical is utilized in several laboratory applications including the isolation of DNA, RNA and proteins.

The entire range of chemicals can be divided into the following categories based upon their applications:

(i) Nucleic acid Extraction and Electrophoresis: Nucleic acid extraction is one of the most basic requirements in Molecular Biology. The diverse nucleic acid extraction protocols are of two categories:

(a) Organic extraction-based Traditional Method

(b) Commercial Column-based Method

(a) Organic extraction-based Traditional Method:

The conventional organic extraction-based alkaline lysis method and the phenol chloroform extraction strategy are very popular because they are inexpensive and do not require state-of-the-art equipments. Conventional methods consist of a lysis procedure to fragment the complex starting material (e.g., blood or tissue) and inactivate the cellular nucleases with preservation of the target nucleic acid. Usually detergents like SDS (MB010), Triton X-100 (MB031) and CTAB (MB101) or denaturants such as urea (MB032), Guanidinium salts (MB014, MB015), and other chaotropes are used for these purpose. Reducing agents [2-Mercaptoethanol (MB041), Dithiothreitol (MB070)] prevent oxidative damage of nucleic acids. Lithium chloride (MB038) is often used specifically for extraction of RNA because Li^+ does not precipitate double-stranded DNA, proteins or carbohydrates. Salt is essential for DNA precipitation because its cations counteract the repulsion caused by the negative charges of the phosphate backbone. Ammonium acetate (MB033) is useful because it is volatile and easily removed and at high concentration it selectively precipitates high molecular weight molecules. Phenol solubilizes and extracts proteins and lipids to the organic phase by sequestering them away from nucleic acids. Phenol titrated to a pH of 8 (MB082) is used to separate DNA from proteins and lipids, since DNA is insoluble in basic phenol. Removal of proteins from nucleic acids can be achieved by extraction with phenol:chloroform solutions (MB078).

(b) Commercial Column-based Method:

To avoid handling of hazardous chemicals like phenol and chloroform and for making the extraction procedure simpler many companies came up with column-based kit. These kits consistently give good yield of DNA which are pure enough for downstream applications. Four simple steps are followed during the extraction procedure: lysis, binding to column, washing of column and elution. The lysis is done with a buffer containing chaotropic salts which include Guanine hydrochloride (MB014), Guanidine thiocyanate (MB015), urea (MB032) and detergents SDS (MB010), Triton X-100 (MB031), Tween 20 (MB067), NP-40 (MB143). The chaotropic salts along with alcohol [Diluent for DNA Extraction (MB228), Isopropanol (MB063)] plays a vital role in binding of the nucleic acids to the silica-based column. Ethanol is used for 1 – 2 times to remove the chaotropic salts which is crucial to get pure DNA or RNA. In the final step, DNA/RNA is eluted from the silica membrane.

The extracted nucleic acids are electrophoresed on agarose gels (refer to the agarose section).

(ii) Protein Extraction, Purification and Electrophoresis: Efficient cell lysis and maximum protein extraction yields are vital to high-quality recombinant protein purification. Detergent-based cell lysis method has become very popular these days. In general, nonionic and zwitterionic detergents are milder, resulting in less protein denaturation upon cell lysis than ionic detergents and are used to disrupt cells when it is critical to maintain protein functions or interactions. CHAPS (MB084), a zwitterionic detergent, and Triton-X (MB031) series of nonionic detergents are commonly used for these purposes. In contrast, ionic detergents are strong solubilizing agents and tend to denature proteins, thereby destroying protein activity and function. SDS (MB010), an ionic detergent that binds to and denatures proteins, is used extensively during protein extraction and purification. During protein extraction procedure, protease inhibitors e.g. Aprotinin (MB119), PMSF (MB144) are used for the instantaneous protection of proteins from various cellular proteases.

Affinity based recombinant protein purification is emerging as purified protein is the basic requirement for proteomics. Protein A Sepharose (MB112) functions as an immunoadsorbent and is widely used for regular purification of antibodies. Glutathione reduced (MB166) and Imidazole (MB019) are used during the elution steps of GST-tagged and Nickel-based affinity chromatog-raphy, respectively.

After the extraction/purification of protein sample, it should be analyzed by electrophoresis. SDS-PAGE (SDS-Polyacrylamide