

Restriction Enzyme Handling

1) **Keep restriction enzymes cold**

Restriction enzymes are proteins and are very sensitive to heat, and freezing and thawing. To keep them cold, they are usually stored at -20 degrees C. To prevent them from freezing, restriction enzymes are usually stored in 50% glycerol.

When out of the freezer always keep restriction enzymes on ice. Never hold a restriction enzyme tube where the enzymes are, because your warm hands will immediately warm them up.

2) **Never contaminate restriction enzyme stocks.**

Always use a fresh sterile tip when going into a restriction enzyme stock. It is simple to ruin an expensive restriction enzyme stock by contaminating it with another restriction enzyme.

3) **Use appropriate buffer conditions for digestions.**

Restriction enzymes work best in a specific ionic environment. Most enzymes are classified by which type of salt solution they work best in (High, Medium or Low). A high salt buffer is referred to as "H". These buffers are in 10X stocks. They are usually buffered between pH 7.4 and 8.2. Each company that makes a restriction enzyme will send a buffer that it works best with.

4) **Use appropriate amount of enzyme**

Restriction enzymes stocks are described in units per volume. One unit of a restriction is usually defined as the amount of enzyme that can digest 1 μg of phage DNA in one hour at 37 degrees C. Since enzyme activity may be lower than expected, it is a good rule of thumb to 2-5X the amount of enzyme that is theoretically required.

5) **Use appropriate volume for digestion**

A good rule of thumb is that the concentration of DNA in the restriction digest should be no more than 100 ng/ μl . Sometimes restriction digestions are inhibited at higher DNA concentrations.

6) Final glycerol concentration must not exceed 5%

Glycerol can affect the sequence specificity of a restriction enzyme at concentrations above 5%. Restriction enzymes are stored in 50% glycerol. Therefore the volume of enzyme added must be no more than 10% of the final volume of the digest.

BSA option

When proteins are diluted they can denature. To keep restriction enzymes more stable, nuclease-free BSA (100 $\mu\text{g/ml}$ final concentration) is often included in restriction digests. BSA (bovine serum albumin) is an inert protein.

DTT option

Restriction enzymes are sensitive to oxidation. To prevent oxidation, reducing agents such as dithiothreitol (DTT) are often included in restriction digests at final concentrations of 1mM to 5mM.