Restriction Endonucleases: Standard Quality Control

Unit Definition

One unit of restriction endonuclease activity is defined as the amount of enzyme required to produce a complete digest of 1 µg of substrate DNA (or fragments) in a total reaction volume of 50 µl in 60 minutes under optimal assay conditions as stated for each restriction endonuclease.

Determination of the volume activity of restriction endonucleases

Restriction endonuclease activity assays are performed by adding different enzyme dilutions to the appropriate assay buffer containing 1 µg of substrate DNA. After a 60-minutes incubation at the appropriate temperature, the digestion is stopped and the DNA samples are visualized by agarose gel/ethidium bromide electrophoresis. The most diluted enzyme solution giving a complete digest is used to calculate the activity in units/ µl.

• Please note that the activity is substrate dependent and when working with a new substrate, the enzyme should be titrated to determine the actual or expected activity.

Quality Controls

The results of all quality control assays are reported on the Technical Data Sheet provided with each enzyme.

Overdigestion Assay

MINOTECH uses an overdigestion assay as a qualitative determination of enzyme purity and of a lack of nonspecific DNases. In the overdigestion assay, increasing amounts of each restriction endonuclease (usually 10, 20, 30, 40, 50 units) are added to a series of tubes containing 1 µg substrate DNA. After a 20-hours incubation under the recommended assay conditions, the maximum number of units giving a clear, sharp, normal banding pattern is determined by agarose gel/ethidium bromide electrophoresis.

To pass the test, the enzyme must yield an unaltered banding pattern under conditions of up to 800-fold overdigestion (units x hours) as compared to a 2-fold digest. If enzyme exhibits “star” activity at a lower than 800-fold functional excess, the product description includes information on the functional excess at which the “star” activity does not occur.

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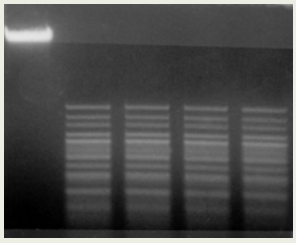

C 1000 2000 3000 4000-fold overdigestion

Assay for Nonspecific Endonucleases

To assay for nonspecific endonuclease contamination, each restriction endonuclease is incubated with a supercoiled plasmid substrate lacking the recognition sequence of the restriction endonuclease. A single nonspecific nick in the RF I DNA converts it to the RF II form (nicked circle). Increasing amounts of enzyme (usually 10, 20, 30, 40, 50 units) are added to a series of tubes containing 1 µg of RF I (supercoiled form) DNA. After a 20-hours incubation under the recommended assay conditions, the two forms are distinguished on agarose gels and the percent conversion from RF I to RF II is determined.

Ligation and Recutting Assay

MINOTECH uses a ligation assay to determine the functional purity of the DNA after restriction enzyme digestion. Substrate DNA is completely digested with a 10- and 50-fold excess of the restriction endonuclease in the appropriate assay buffer, ligated with T4 DNA Ligase and recut with the same restriction enzyme. Cut, ligated and recut DNAs are analyzed by agarose gel/ethidium bromide electrophoresis. A normal banding pattern indicates intact 5´ and 3´ termini as well as the absence of contaminating nucleases or phosphatases.

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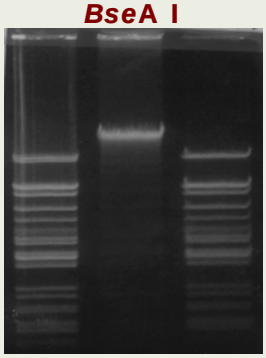

Cut Ligation Recut

Stability

All MINOTECH restriction endonucleases are reassayed every 4-6 months. This process allows us to ensure full enzyme activity and optimal performance in every enzyme we ship. Due to the excellent results of this testing, we have extended the expiration dates of most of our enzymes to 24 months.

All the enzymes must be stored at -20^{O}C.