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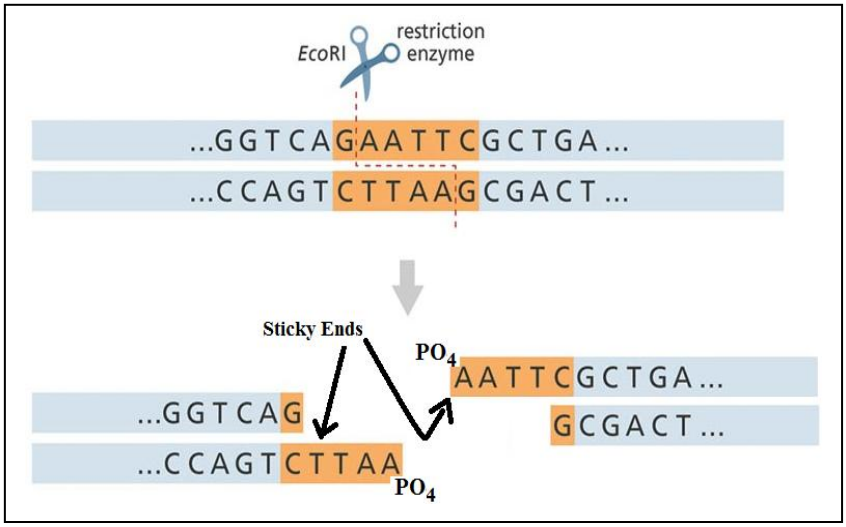
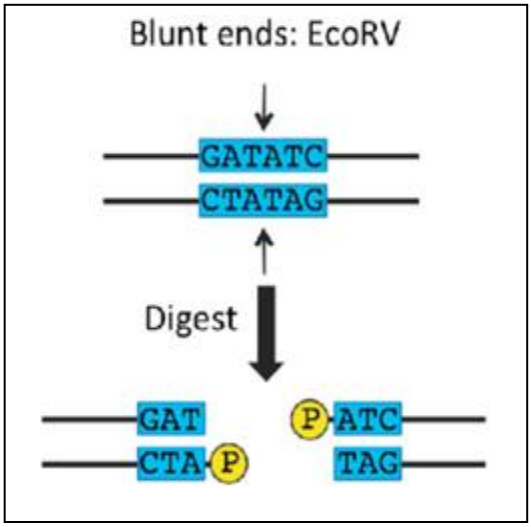
Product Name	Cat. No.	Pack Size
Restriction Digestion KIT	**# BB-MTK040S**	**5 Reactions**

- A **restriction enzyme** or **restriction endonuclease**, or restrictase is an **enzyme** that cleaves double stranded DNA into fragments at or near specific recognition sites within that DNA molecules.
- These **enzymes** are found in bacteria and archaea and provide a defense mechanism against invading viruses.

Over 3,000 restriction enzymes are now known and more than 600 of restriction enzymes are commercially available for routine use in laboratories as a vital tool in molecular cloning for DNA modifications.

Restriction enzymes are primarily used in the laboratory for generating 1) Sticky Ends and 2) Blunt Ends DNA molecules for cloning purposes.





Isochizomers:
Any restriction endonuclease will cut only a specific base sequence, no matter what DNA molecule it is acting on. However, a given recognition sequence can be recognized by multiple enzymes called **isochizomers** e.g., *Sma* I and *Xma* I. These can cleave the DNA at same or different position within the recognition sequence.

Factors affecting Restriction Enzyme Activity:

Buffer Systems: Tris-HCl is the most commonly used buffering agent in incubation mixtures, which is temperature dependent. Most restriction enzymes are active in the pH range 7.0 - 8.0.

Temperature: Most digestions are carried out at 37°C. However, there are a few exception e.g., digestion with *Sma*I is carried out at lower temperatures (~25°C), while with *Taq* I at higher temperature i.e., 65°C.

Ionic Conditions: Mg2+ is an absolute requirement for all restriction endonucleases, but the requirement of other ions (Na+/K+) varies with different enzymes.

Methylation of DNA: Methylation of specific adenine orcytidine residues within the recognition sequence of the restriction enzyme affects the digestion of DNA.

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Restriction Digestion KIT

Kit description:

Using this kit, students will perform restriction enzyme digestion of the supplied plasmid DNA having 11,334 base pairs (bp) with two different enzymes, *Hin*d III and Sal I. It has recognition sites for many restriction enzymes. Enzymes supplied in this kit, *Sal* I and *Hin*d III have 2 and 7 recognition sequences on this supplied plasmid DNA. On Digestion with *Sal* I and *Hin*d III, two and seven fragments of different sizes are obtained respectively. These bands are then resolved by Agarose gel electrophoresis to observe the different restriction enzyme patterns. Simultaneously, a molecular weight ladder will also be electrophoreses to assess the various fragment sizes. (P*lease see the agarose gel electrophoresis profile of the digested DNA at the last page).*

Enzyme	Fragment length (bp)
Hind III	4027bp, 2866bp, 1678bp, 1322bp, 715bp, 445bp, 218bp
Sal I	8649bp, 2685bp

Materials Provided (For 5 Reactions only):

The list below provides information about the materials supplied in the kit. The products should be stored as suggested. Use the kit within 3 months of arrival.

Materials	Quantity	Store
Plasmid DNA(50 ng/µl)	50 µl	-20°C
10X Assay Buffer	25 µl	-20°C
HinDIII Enzyme	6.25 µl	-20°C
Sal I Enzyme	6.25 µl	-20°C
1 Kbp DNA Ladder	25 µl	-20°C
Control DNA	25 µl	-20°C
Agarose	2 gm	RT
6 X DNA Dye	25 µl	4°C
50 X TAE Buffer	15 ml	4°C
1.5 ml Tube	20 Nos.	RT
*Ethidium Bromide ***(Take precautionary measure while handling as it is a potent carcinogen)***	12.5 µl (Concentration: 10 mg / ml)	RT
Nuclease Free Water	1 ml	RT

Setting up the Restriction Digestion Reaction:

1. Place the vials containing restriction enzyme (*Hin*d III and Sal I) on ice.

2. Thaw the vials containing substrate (Plasmid DNA) and Reaction buffer.

3. Prepare two different reaction mixtures using the following constituents.

Reaction 1 (*Hin*d III digestion)

Plasmid DNA	5µl
Reaction Buffer	2 µl
Hind III Enzyme	1 µl
Nuclease Free Water	12 µl
Total Reaction Mixture	20 µl

Reaction 2 (*Sal* I digestion)

Plasmid DNA	5µl
Reaction Buffer	2 µl
Sal I Enzyme	1 µl
Nuclease Free Water	12 µl
Total Reaction Mixture	**20 µl**

4. Incubate the vial at 37°C for 2 hour.

5. Meanwhile, prepare a 0.7% agarose gel for electrophoresis. *(Please follow the direction at the last page).*

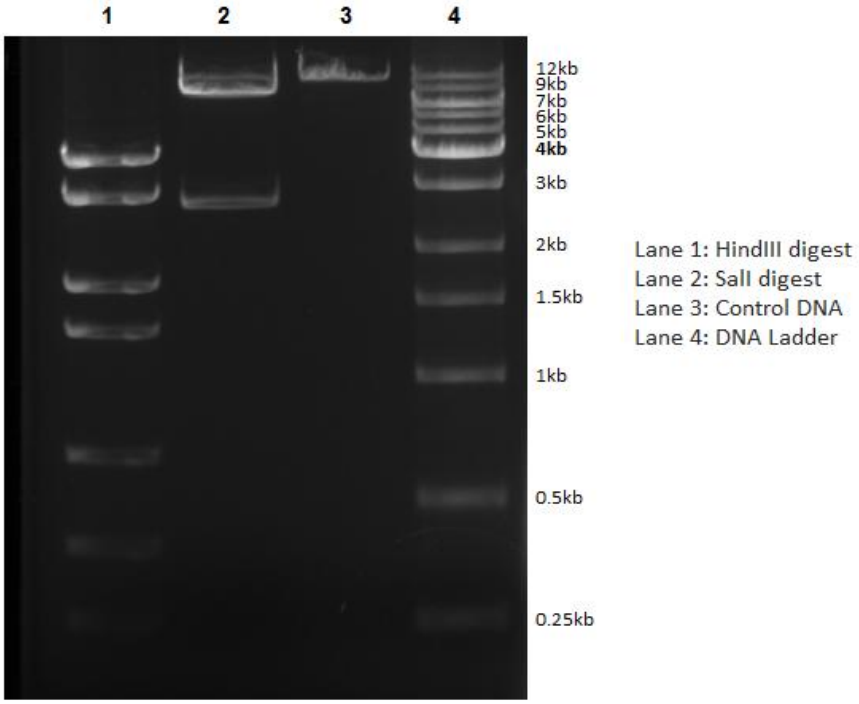
6. After 2 hours add 5 µl of gel loading Dye to each vials.

7. Load the digested samples, 5 µl of Control DNA and 5 µl of DNA Ladder, note down the order of loading.

8. Electrophoreses the samples at 50-100 V for 1-2 hours.

Restriction Digestion KIT

Observation:



Interpretation:

Restriction patterns obtained on digestion with *Hin*d III and *Sal* I are markedly different, demonstrating the fact that each restriction enzyme recognizes and cuts only a particular base sequence unique to it. By comparing the migration distances with that of the marker, one can also determine the approximate sizes of DNA fragments.

Preparation of 0.7% Agarose gel electrophoresis:

- Prepare 0.7% agarose solution in 50 X TAE buffer provided (e.g. for 50 ml of agarose gel add 0.35 gm of agarose, 1 ml of TAE & 49 ml of sterile water).
- Boil until the agarose is completely dissolved.
- After boiling let the sol. to be cooled down around 37°C - 40°C
- Add 2.5 µl of Ethidium Bromide from the supplied stock (**very carefully**) & mix properly.
- Seal the gel casting tray on two sides; place the comb in the gel tray in appropriate position.
- Pour the agarose mixture in the tray containing comb.
- Let the agarose to be solidified in the tray. Remove the seal from the two sides without disturbing the gel.
- Next keeps the gel tray in the tank containing 1X TAE buffer. Keep the wells of the gel in the cathode (Negative) side. The buffer level in the tank should be maintained above the gel tray.
- Lift the comb from the gel gently to avoid damaging of wells; the gel is now ready for loading.
- Connect the cords of electrophoresis tank with the power pack before loading the samples.
- To prepare samples for electrophoresis add 3-5 µl of 6X gel loading dye into the sample & mix well by pipetting or pulse centrifugation. Load 20 µl of sample into the well.
- Then switch on the power pack & adjust the voltage in between 60V to 100V.
- Continue the electrophoresis until the dye reaches to1/3rd or above of the gel.