

Restriction Digest Protocol

Materials

- 10X NEBuffer (standard or unique)
- 6X Purple Load Dye (may be supplied)
- Additives if needed:
 - Adenosine 5'-Triphosphate (ATP) (supplied when required)
 - rAlbumin (supplied when required)
 - DTT (supplied when required)
 - PaqCI Activator (supplied when required)

Materials required but not supplied:

- DNA
- Nuclease-free Water ([NEB #B1500](#))

Overview

Restriction enzyme digests require proper quantities of DNA, enzyme, and buffer, in an appropriate reaction volume ratio. For molecular cloning, the “typical” reaction conditions below use 5-10x over digestion to compensate for variabilities in DNA sample quality and quantity. [Time-Saver™ qualified restriction enzymes](#) and [High-Fidelity \(HF®\) restriction enzymes](#) perform under a broader range of recommended conditions. Critically, individual restriction endonuclease recommended reaction times and temperatures can vary significantly.

NEBcloner® tool is strongly recommended to design reactions for successful digests.



Additional information to optimize restriction enzyme digests can be found in our usage guidelines: [Optimizing Restriction Endonuclease Reactions](#) and [Restriction Enzyme Tips](#).

Reaction set-up:

Set up the following reaction as follows on ice.

(Please note that enzymes should always be added last.)

COMPONENTS	50 µl REACTION	FINAL CONC. OR AMOUNT
DNA	1 µg	
10X NEBuffer**	5 µl	1X
Restriction Enzyme	1µl	
Nuclease-free Water	to 50 µl	Varies*

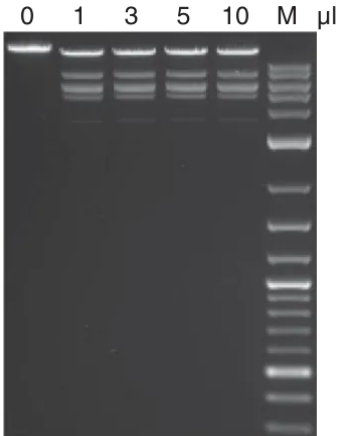
**We recommend 5-10 units of enzyme per ug of DNA. Adjust according to protocol at [NEBcloner.neb.com](https://www.neb.com). For convenience, 1µl can be used.*

***Additive (such as DTT, Activator or ATP) may be required for certain enzymes.*

Digest protocol:

1. Gently mix the reaction by pipetting up and down and microfuge briefly.
2. Incubate at temperature and time ranges recommended for the specific restriction endonuclease.
3. Stop the reaction.
 - If no further manipulation of DNA is required, use 10 µl per 50 µl reaction of stop solution containing EDTA,(e.g. [Gel Loading Dye, Purple \(6X\)](#), [Gel Loading Dye, Purple \(6X\)](#), no SDS, or [Gel Loading Dye, Orange \(6X\)](#))

Gel with restriction enzyme digested Lambda DNA



50 µl digestion reactions were set up using a range of amounts of [EcoRI-HF®](#) restriction enzyme, 1 µg of Lambda DNA, and the recommended reaction buffer, resulting in no star activity in overnight digests at 37°C, even when used at higher concentrations. Marker M is the 1 kb DNA Ladder ([NEB# N3232](#)). The [Monarch Spin DNA Gel Extraction Kit \(NEB #T1120\)](#) is recommended for DNA gel extraction and purification

General Guidelines

1. If further manipulation of DNA is required, use either heat inactivation at the appropriate temperature (65°C or 80°C) for 20 minutes (please note not all enzymes are inactivated by heat), phenol/chloroform extraction, or spin column purification (e.g. [Monarch® Kits for DNA Cleanup](#)) to remove the enzyme.
2. For highly concentrated DNA for downstream applications, use the Monarch Spin DNA Gel Extraction Kit ([NEB #T1120](#)) to attain high-quality DNA purification with low-elution volumes.

Related Resources

- [Optimizing Restriction Endonuclease Reactions](#)
- [Restriction Enzyme Tips](#)
- [NEBcloner®](#)
- [NEBcutter®](#)
- [NEBioCalculator®](#)
- [REBASE®](#)
- [Double Digest Finder](#)
- [Enzyme Finder](#)
- [Competitor Cross-Reference Tools](#)