

[PRODUCTS](#)[TECHNICAL
REFERENCE](#)[CUSTOMER
SERVICE](#)[MY NEB
ACCOUNT](#)[search](#)[CONTACT NEB](#)[ABOUT US](#)[SITE MAP](#)[REQUEST A CATALOG](#)[INTERNATIONAL ORDERS](#)[FREEZER PROGRAM](#)[QUICK ORDER](#)[Home](#) > [Technical Reference](#) > [Restriction Endonucleases](#) > [Double Digests](#)

Double Digests

Cleaving a DNA substrate with two restriction endonucleases simultaneously (double digestion) is a common timesaving procedure.

Selecting the best NEBuffer to provide reaction conditions that are amenable to both restriction endonucleases is an important consideration. Each enzyme is supplied with its optimal NEBuffer to ensure 100% activity. NEBuffer compositions are listed on [buffer pages](#) and on the data card sent with each enzyme. The [NEBuffer Activity Chart](#) rates the percentage activity of each restriction endonuclease in the four standard NEBuffers. The NEBuffer that results in the most activity for both enzymes should be used for the double digest.

Depending on an enzyme's activity rating in a non-optimal NEBuffer, the number of units of enzyme or incubation time of the reaction may be adjusted to compensate for slower rate of cleavage.

If no single NEBuffer can be found to satisfy the buffer requirements of both enzymes, the reactions can be done sequentially (seq). First, cleave with the restriction endonuclease that requires the lower salt reaction conditions, then adjust the salt concentration of the reaction (using a small volume of a concentrated salt solution) to approximate the reaction conditions of the second restriction endonuclease. Add the second enzyme and incubate to complete the second reaction.

Performing double digests with enzymes that are supplied with their own unique (designated "U") NEBuffer and not one of the four standard NEBuffers is also simple. In most cases, double digests using any of these enzymes with an enzyme that is supplied with one of the four standard NEBuffers can be done in the unique NEBuffer. This will ensure that the enzyme with the more specific buffer requirements will work optimally. When using restriction endonucleases in non-optimal NEBuffers, more enzyme or longer digestion time may be needed to compensate for the slower rate of cleavage under those conditions. Check the [NEBuffer Activity Chart](#) to see how well the second enzyme performs in the salt range of the unique NEBuffer.

Suggested NEBuffers for Double Digestion

ENZYME	Aat II	Avr II	BamHI	Bgl II	Bsg I	Eag I	EcoRI	EcoR V	Hind III	Kpn I	Mse I	Nco I	Nde I	Nhe I	Not I	Pst I	Pvu I	Sac I	Sac II	Sal I	Sma I	Spe I	Sph I	Xba I	Xho I
NEBuffer	4	2	U	3	4	3	U	3	2	1	2	4	4	2	3	3	3	1	4	3	4	2	2	2	2
Avr II	2	4																							
BamH I	U	seq	seq																						
Bgl II	3	seq	3	BamHI																					
Bsg I	4	4	4	seq	4																				
Eag I	3	seq	3	BamHI	3	seq																			
EcoRI	U	4	EcoRI	EcoRI	EcoRI	4	EcoRI																		
EcoR V	3	4	2	BamHI	3	4	3	EcoRI																	
Hind III	2	4	2	seq	2	2	seq	EcoRI	2																
Kpn I	1	4	1	seq	2	seq	seq	2	2	2															
Mse I	2	4	2	seq	2	2	2	EcoRI	2	2	1														
Nco I	4	4	2	BamHI	3	4	3	EcoRI	3	2	1	2													
Nde I	4	4	4	BamHI	3	4	3	EcoRI	2	2	1	2	4												
Nhe I	2	4	2	seq	2	4	2	EcoRI	2	2	1	2	2	4											
Not I	3	4	3	BamHI	3	3	3	EcoRI	3	2	2	2	3	3	2										
Pst I	3	4	3	BamHI	3	3	3	EcoRI	3	2	1	2	3	3	2	3									
Pvu I	3	seq	3	BamHI	3	3	3	EcoRI	3	2	2	3	3	3	2	3	3								
Sac I	1	4	1	seq	2	1	seq	seq	2	2	1	2	1	4	1	4	1	2							
Sac II	4	4	2	BamHI	seq	4	seq	EcoRI	2	2	1	2	4	4	4	4	2	seq	4						
Sal I	3	seq	seq	BamHI	3	seq	3	EcoRI	3	seq	seq	3	3	3	seq	3	3	seq	seq						
Sma I	4	4	4	seq	seq	4	seq	seq	4	4	seq	2	4	4	4	4	4	seq	4	4	seq				
Spe I	2	4	2	BamHI	2	2	3	EcoRI	2	2	1	2	2	2	2	2	2	2	1	2	seq	4			
Sph I	2	4	2	BamHI	2	4	3	EcoRI	2	2	1	2	2	2	2	2	2	2	1	4	seq	4	2		
Xba I	2	4	2	seq	2	2	3	2	2	2	2	2	2	2	3	3	3	4	2	3	4	2	2		
Xho I	2	4	2	BamHI	3	4	3	EcoRI	3	2	1	2	2	4	2	3	3	1	2	3	4	2	2	2	
Xma I	4	4	4	seq	seq	4	seq	seq	4	seq	4	4	4	4	4	4	seq	4	4	seq	4	4	4	4	4

Double Digest Notes:

- If BSA is a buffer requirement for either enzyme, add it to the double digest reaction. BSA will not inhibit any restriction enzyme.
- The final concentration of glycerol in any reaction should be less than 5% to minimize the possibility of star activity. To avoid this situation, an increase in total reaction volume may be necessary.
- Double digestion is not recommended for certain enzyme combinations. In these cases a sequential digest is required. In the table above, these combinations are indicated by the abbreviation "seq".
- This data is based on 1-2 hour digests. Overnight double digests should be avoided due to possible star activity.
- Bsp I requires SAM. SAM as an additive did not have a negative affect on activity of other enzymes.
- Sma I is incubated at 25°C; then raise the temperature to 37°C to allow your second enzyme to work. Sma I may be heat killed between the digests.

[Privacy](#), [Limitations](#), [Warranty](#), [Disclaimer](#), [Copyright](#) and [Trademark](#)