

## Double Digestion of DNA with *mi* Splendid - Restriction Enzymes (02.03.2007)

Simultaneous cleavage of DNA with two different restriction endonucleases is a common time-saving procedure. The best buffer can be chosen following the recommendations of *metabion* Enzyme Activity Chart which rates the activity of each restriction endonuclease in the 5 different buffers.

In the table below you will find recommended buffers for double digestions using 15 of the most common restriction endonucleases. If no single *metabion* buffer 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 to approximate the reaction conditions of the second restriction endonuclease. Add the second enzyme and incubate to complete the second reaction.

When using restriction endonucleases in non-optimal buffers, more enzyme or longer digestion time may be needed to compensate for the slower rate of cleavage under those conditions.

### Suggested Buffers for Double Digestion

	<i>Bam</i> H I	<i>Bgl</i> II	<i>Eco</i> R I	<i>Eco</i> R V	<i>Hind</i> III	<i>Kpn</i> I	<i>Nco</i> I	<i>Not</i> I	<i>Pst</i> I	<i>Pvu</i> II	<i>Sal</i> I	<i>Sla</i> I	<i>Sma</i> I	<i>Sph</i> I	<i>Sst</i> I	
	U	B3	U	B2	B2	U	B3*	U	U	B2	B4	B4	B5	B2	B1	
<i>Bgl</i> II	B3	B3														
<i>Eco</i> R I	U	<i>Eco</i> R I	<i>Eco</i> R I													
<i>Eco</i> R V	B2	B2	B2	<i>Eco</i> R I												
<i>Hind</i> III	B2	B2	B2	<i>Eco</i> R I	B2											
<i>Kpn</i> I	U	seq	B2	seq	B2	<i>Kpn</i> I/B2										
<i>Nco</i> I	B3*	<i>Bam</i> H I	B3	<i>Eco</i> R I	B2	B2	B1									
<i>Not</i> I	U	<i>Bam</i> H I	B3	<i>Eco</i> R I	B3	B2	seq	B4*								
<i>Pst</i> I	U	<i>Bam</i> H I	B3	<i>Eco</i> R I	B2/B3	B2	seq	B3	B3							
<i>Pvu</i> II	B2	<i>Bam</i> H I	B3	<i>Eco</i> R I	B2	B2	B1	B3	B3	B3						
<i>Sal</i> I	B4	B4	B4	<i>Eco</i> R I	B3	seq	seq	B4	B4	B4	B3					
<i>Sla</i> I	B4	<i>Bam</i> H I	B3	<i>Eco</i> R I	B2	B2	seq	B4	B4	B3	B3	B4				
<i>Sma</i> I	B5	B5	seq	seq	B5	B5	B5	B5	B5	B5	B5	seq	seq			
<i>Sph</i> I	B2	B2	B2	<i>Eco</i> R I	B2	B2	B1	B2	B4*	B2	B2	B4	B2	B5		
<i>Sst</i> I	B1	B1	B2	seq	B2	B2	B1	B1	B5	B5	B5	seq	seq	B5	B1	
<i>Xba</i> I	B2	B2	B2/B3	<i>Eco</i> R I	B2	B2	B1	B2	B4*	B3	B2	B4	B2/B4	B5	B2	B1

All the reactions were carried out in the presence of BSA (100 µg/ml). Our experience indicates that it is important to use BSA in reaction mixtures in order to obtain successful digestions of DNA. The presence of BSA gives complete and reproducible cleavages for a range of DNA substrates. BSA stabilizes the enzymes when digestions are performed for more than one hour at 37 °C, since many restriction endonucleases in reaction buffers without BSA can survive at this temperature for 10-20 minutes only or even less. Also, BSA binds metal ions, and other chemicals, which might be present in buffers or DNA preparations, thereby inactivating restriction endonucleases.

**Note:** BSA is not included into all supplied reaction buffers and should be added separately from the supplied stock solution (1 mg/ml). Please have a look into the corresponding data sheet!

- Requires Triton X-100 for optimal activity. TX-100 is included into the supplied reaction buffer.
- The following enzymes can exhibit "star" activity: *Bam*H I, *Bcl* I, *Bse*B I, *Bss*A I, *Eco*R I, *Eco*R V, *Hind* III, *Hpa* I, *Kpn* I, *Nco* I, *Nru* I, *Pst* I, *Pvu* II, *Sal* I, *Sca* I, *Sna*B I, *Sph* I, *Ssp* I, *Xba* I.

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