Reaction Buffers for OPTIZYME™ Restriction Enzymes

Part No.	OPTIZYME* Buffer	1X OPTIZYME* Buffer Composition											pH @ 37 C
		Tris-HCl, mM	MgCl2, mM	NaCl, mM	KCl, mM	BSA, mg/ml	Tris-Acetate, mM	Mg-Acetate, mM	K-Acetate, mM	Bis-Tris Propane-HCl, mM	Sodium Glutamte, mM	Triton X-100, %	
BP8084-1	10X Buffer 1	10	10			0.1							7.5
BP8085-1	10X Buffer 2	10	10	50		0.1							7.5
BP8086-1	10X Buffer 3	50	10	100		0.1							7.5
BP8087-1	10X Buffer 4					0.1	33	10	66				7.9
BP8088-1	10X Buffer 5	10	10		100	0.1							8.5
BP8096-1	10X Buffer AarI, ScaI, PasI		10		100	0.1				10			6.5
BP8089-1	10X Buffer BamHI	10	5		100	0.1						0.02	8.0
BP8093-1	10X Buffer Cfr9I	10	5			0.1					200		7.2
BP8095-1	10X Buffer Ecl136II, SacI		10			0.1				10			6.5
BP8090-1	10X Buffer EcoRI	50	10	100		0.1						0.02	7.5
BP8094-1	10X Buffer KpnI	10	10			0.1						0.02	7.5
BP8091-1	10X Buffer Sau3AI					0.1	33	10	66			0.02	7.9
BP8092-1	10X Buffer TaqI	10	5	100		0.1							8.0

When combining OPTIZYME* restriction enzymes with restriction enzymes from other suppliers to perform double DNA digests, compare buffer compositions of the OPTIZYME* buffer with the buffer from another supplier.

If the buffers are somewhat compatible (i.e., similar Tris-HCl or salt concentration),

1. Perform digest with the first enzyme in a small volume (20µl) at the recommended temperature and incubation time.
2. Perform the second digest sequentially at the optimal temperature and incubation time. Bring reaction volume up to 100ul to dilute out the first buffer (ensure that glycerol concentration is ≤5% to minimize star activity). If the second enzyme has reduced activity in the first enzyme buffer, increase the number of units used for the second enzyme.

If buffers are completely incompatible,

1. Perform digest with the first enzyme at the recommended temperature and incubation time.
2. Heat inactivate the first enzyme at the recommended temperature. Purify the digested DNA by spin column or by phenol/chloform/IAA and then EtOH precipitation.
3. Perform the second digest (sequentially) at the optimal temperature and incubation time.