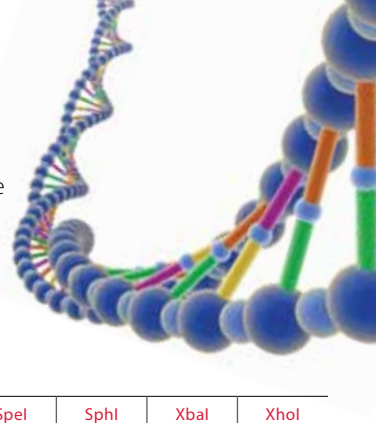
Recommended universal buffers for double digestion



Double digestion (cutting DNA with two restriction enzymes simultaneously) is widely used to save time. In this table, the suitable buffer type, dilution rate, and additive are shown to perform double digestion with the restriction enzymes shown below. The letters L, M, H, T, and K show the type of universal buffer recommended, which is supplied at a 10-fold (10X) concentration. When "0.5X" is recommended, dilute 10X buffer 20-fold; when "1X" is recommended, dilute 10-fold; and when "2X" is recommended, dilute 5-fold for use in double digestion. As BSA is also supplied at 10X concentration, dilute 10-fold to a final concentration of 0.1% when it is used.

Enzyme	AccI	BamHI	BglII	ClaI	EcoRI	EcoRV	HincI	HindIII	KpnI	NcoI	NdeI	NotI	PstI	PvuI	SacI	SalI	SmaI	SpeI	SphI	XbaI	XhoI
Supplied buffer	10X M	10X K	10X H	10X M	10X H	10X H	10X M	10X M	10X L	10X K +BSA	10X H	10X H +BSA +Triton	10X H	10X K +BSA	10X L	10X H	10X T +BSA	10X M	10X H	10X M +BSA	10X H
AccI	–	0.5X K	1X T	1X M	1X M	0.5X K	1X M	1X M	1X M	1X M +BSA	1X T	0.5X K +BSA	1X M	0.5X K	1X M	1.5X T	1X T +BSA	1X M	0.5X K	1X M	1X M
BamHI	0.5X K	–	1X K	1X K	1X K	1X K	0.5X K	1X K	0.5X K	1X K +BSA	1X K	0.5X K +BSA	1X K	1X K	0.5X K	1.5X T	0.5X T +BSA	1X K	1X K	0.5X K	1X M
BglII	1X T	1X K	–	1X H	1X H	1X H	2X K	1X K	1X T	1X K +BSA	1X H	1X H +BSA	1X H	1X K	0.5X K	1X H	1X T +BSA	1X H	1X H	2X T	1X H
ClaI	1X M	1X K	1X H	–	1X H	1X H	1X M	1X M	1X M	1X K +BSA	1X H	1X H +BSA	1X H	1X K	1X M	1X H	1X T +BSA	1X M	1X H	1X H	1X H
EcoRI	1X M	1X K	1X H	1X H	–	1X H	1X M	1X M	1X M	1X K +BSA	1X H	1X H +BSA	1X H	1X K	1X M	1X H	1X T +BSA	1X H	1X H	1X M	1X H
EcoRV	0.5X K	1X K	1X H	1X H	1X H	–	2X T	1X K	0.5X K	1X K +BSA	1X H	1X H +BSA	1X H	1X K	0.5X K	1X H	0.5X K +BSA	1X H	1X H	2X T	1X H
HincII	1X M	0.5X K	2X K	1X M	1X M	2X T	–	1X M	1X M	1X M +BSA	1X T	0.5X K +BSA	1X M	0.5X K	1X M	1.5X K	1X T +BSA	1X M	2X T	1X M	1X M
HindIII	1X M	1X K	1X K	1X M	1X M	1X K	1X M	–	1X M	1X K +BSA	1X K	0.5X K +BSA	1X M	1X K	1X M	1.5X K	1X T +BSA	1X M	1X K	1X M	1X M
KpnI	1X M	0.5X K	1X T	1X M	1X M	0.5X K	1X M	1X M	–	0.5X K +BSA	1X T	0.5X K +BSA	1X M	0.5X K	1X L	1.5X T +BSA	1X T +BSA	1X M	0.5X K	1X M	1X M
NcoI	1X M +BSA	1X K +BSA	1X K +BSA	1X K+BSA	1X K +BSA	1X K +BSA	1X M +BSA	1X K +BSA	0.5X K +BSA	–	1X K +BSA	0.5X K +BSA	1X K +BSA	1X K +BSA	0.5X K +BSA	1.5X T +BSA	1X T +BSA	1X K +BSA	1X K +BSA	1X M +BSA	1X K +BSA
NdeI	1X T	1X K	1X H	1X H	1X H	1X H	1X T	1X K	1X T	1X K +BSA	–	1X H +BSA	1X H	1X K	1X T	1X T +BSA	1X H	1X H	1X T	1X H	1X H
NotI	0.5X K +BSA	0.5X K +BSA	1X H +BSA	1X H+BSA	1X H +BSA	1X H +BSA	0.5X K +BSA	0.5X K +BSA	0.5X K +BSA	0.5X K +BSA	1X H +BSA	–	1X H +BSA	2X K +BSA	0.5X K +BSA	1X H +BSA	0.5X T +BSA	1X H +BSA	1X H +BSA	0.5X K +BSA	1X H +BSA
PstI	1X M	1X K	1X H	1X H	1X H	1X H	1X M	1X M	1X M	1X K +BSA	1X H	1X H +BSA	–	1X K	1X M	1X H	0.5X T +BSA	1X H	1X H	1X M	1X H
PvuI	0.5X K	1X K	1X K	1X K	1X K	1X K	0.5X K	1X K	0.5X K	1X K +BSA	1X K	2X K +BSA	1X K	–	0.5X K	1.5X K +BSA	1X K +BSA	1X K	1X K	0.5X K	1X K
SacI	1X M	0.5X K	0.5X K	1X M	1X M	0.5X K	1X M	1X M	1X L	0.5X K +BSA	1X T	0.5X K +BSA	1X M	0.5X K	–	1.5X T +BSA	1X T +BSA	1X M	0.5X K	1X M	1X M
SalI	1.5X T	1.5X T	1X H	1X H	1X H	1X H	1.5X K	1.5X K	1.5X T +BSA	1.5X T +BSA	1X H	1X H +BSA	1X H	1.5X K +BSA	1.5X T +BSA	–	1.5X T +BSA	1X H	1X H	1.5X T	1X H
SmaI	1X T +BSA	0.5X T +BSA	1X T +BSA	1X T +BSA	1X T +BSA	0.5X K +BSA	1X T +BSA	1X T +BSA	1X T +BSA	1X T +BSA	1X T +BSA	0.5X T +BSA	0.5X T +BSA	1X K +BSA	1X T +BSA	1.5X T +BSA	–	1X T +BSA	0.5X T +BSA	1X T +BSA	1X T +BSA
SpeI	1X M	1X K	1X H	1X M	1X H	1X H	1X M	1X M	1X M	1X K +BSA	1X H	1X H +BSA	1X H	1X K	1X M	1X H	1X T +BSA	–	1X H	1X M	1X H
SphI	0.5X K	1X K	1X H	1X H	1X H	1X H	2X T	1X K	0.5X K	1X K +BSA	1X H	1X H +BSA	1X H	1X K	0.5X K	1X H	0.5X T +BSA	1X H	–	2X T	1X H
XbaI	1X M	0.5X K	2X T	1X M	1X M	2X T	1X M	1X M	1X M	1X M +BSA	1X T	0.5X K +BSA	1X M	0.5X K	1X M	1.5X T	1X T +BSA	1X M	2X T	–	1X M
XhoI	1X M	1X K	1X H	1X H	1X H	1X H	1X M	1X M	1X M	1X K +BSA	1X H	1X H +BSA	1X H	1X K	1X M	1X H	1X T +BSA	1X H	1X H	1X M	–

Note:

1. It is confirmed that 10 units of each enzyme completely digests 1 µg of DNA at 37°C in one hour in 50 µL reaction mixture.
2. The concentration of glycerol should be less than 10% to minimize star activity.
3. DNA may not be digested completely when recognition sequences of two enzymes are close to each other or when DNA takes high-structure conformation.

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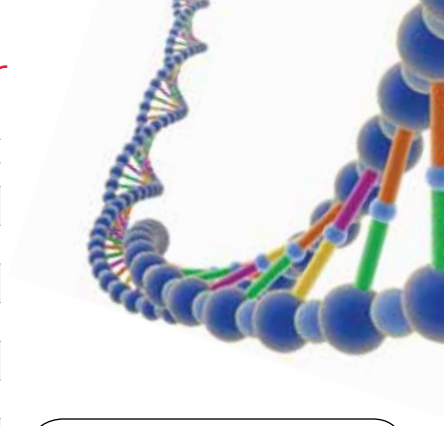
Relative activity for each reaction buffer

Restriction enzyme	Cut site	Relative activity (%) Buffer L	Buffer M	Buffer H	Buffer K	Buffer T (+BSA)	Buffer
AccI	5′-GT↓(Py)(Pu) AC-3′ 3′-CA (Pu)(Py)↑TG-5′	20	**100**	<20	<20*	160	–
AfaI (RsaI)	5′-GT↓AC-3′ 3′-CA↑TG-5′	60	60	40	60	**100**	–
AluI	5′-AG↓CT-3′ 3′-TC↑AG-5′	**100**	100	<20	40	200	–
ApaI	5′-GGGCC↓C-3′ 3′-C↑CCGGG-5′	**100**	<20	<20	<20	<20	–
BalI	5′-TGG↓CCA-3′ 3′-ACC↑GGT-5′	20*	20*	<20*	<20*	40*	**100** (Ball buffer)
BamHI	5′-G↓GATC C-3′ 3′-C CTAG↑G-5′	<20*	<20	40	**100**	<20*	–
BglII	5′-A↓GATC T-3′ 3′-T CTAG↑A-5′	<20	20	**100**	100*	60*	–
BmeT120I (AvaI)	5′-C↓(Py)C G(Pu) G-3′ 3′-G (Pu)G C(Py)↑C-5′	<20	<20	20	**100**	<20	–
BshTI (AgeI)	5′-A↓AGCTT-3′ 3′-TT CGA↑A-5′	<20	20	**80***	50	50	–
ClaI	5′-AT↓CGAT-3′ 3′-TAGC↑TA-5′	40	**100**	120	100	60	–
DdeI	5′-C↓TNA G-3′ 3′-G ANT↑C-5′	60	80	**100**	100	80	–
DpnI	5′-GmA↓ TC-3′ 3′-C T↑mAG-5′	60	60	120	140	**100***	–
DraI (AhaIII)	5′-TTT↓AAA-3′ 3′-AAA↑TTT-5′	100	**100**	60	100	80	–
EcoRI	5′-G↓AATT C-3′ 3′-C TTAA↑G-5′	20*	100*	**100**	120*	80*	–
EcoRV	5′-GAT↓ATC-3′ 3′-CTA↑TAG-5′	<20*	40*	**100**	120*	40*	–
*Eco*T22I (Ava II)	5′-ATGCA↓T-3′ 3′-T↑ACGTA-5′	<20	20	**100**	140*	20*	–
HaeIII	5′-GG↓CC-3′ 3′-CC↑GG-5′	60	**100**	100	60	100	–
HapII (HpaII)	5′-C↓CG G-3′ 3′-G GC↑G-5′	**100**	60	<20	<20	100	–
HhaI	5′-GCG↓C-3′ 3′-C↑GCG-5′	80	**100**	100	120	120	–
HincII (HindII)	5′-GT(Py)↓(Pu)AC-3′ 3′-CA(Pu)↑(Py)TG-5′	20	**100**	20	40	100	–
HindIII	5′-A↓AGCT T-3′ 3′-T TCGA↑A-5′	60*	**100**	<20	200	100*	–
HinfI	5′-G↓ANT C-3′ 3′-C T-↑G-5′	80	100	**100**	160	60	–
HpaI	5′-GTT↓AAC-3′ 3′-CAA↑TTG-5′	<20	40*	20	**100**	80*	–
KpnI	5′-GGTAC↓C-3′ 3′-C↑CATGG-5′	**100**	60	<20	<20	100*	–
MboI (Sau3AI)	5′-↓GATC-3′ 3′-CTAG↑-5′	20	40	60	**100**	40	–
MluI	5′-A↓CGCGT-3′ 3′-TGCGC↑A-5′	60	60	**100**	100*	60	–
MseI	5′-T↓TA A-3′ 3′-A AT↑T-5′	40	40	20	40	**100**	–
NcoI	5′-C↓CATGG-3′ 3′-GGTAC↑C-5′	40*	60*	20	**60***	60*	–
NdeI	5′-CA↓TATG-3′ 3′-GTAT↑AC-5′	<20	40	**100**	10	80	–
NheI	5′-G↓CTAGC-3′ 3′-CGATC↑G-5′	120*	**100**	<20	<20	160*	–
NotI	5′-GC↓GGCCGC-3′ 3′-CGCCGG↑CG-5′	<20*	<20*	**20****	<20	<20*	–
NruI	5′-TCG↓CGA-3′ 3′-AGC↑GCT-5′	0*	<20*	20*	20*	<20*	**100** (NrUI buffer)
PstI	5′-C TGCA↓G-3′ 3′-G↑ACGT C-5′	<20*	60*	**100**	80	20*	–
PvuI	5′-CGAT↓CG-3′ 3′-GC↑TAGC-5′	<20*	20*	40*	**80****	40*	–
PvuII	5′- CAG↓CTG-3′ 3′- GTC↑GAC-5′	80*	**100**	40	<20	40*	–
SacI	5′-GAGCT↓C-3′ 3′-C↑TCGAG-5′	**100**	60	<20	<20	80	–
SacII	5′- CCGC↓GG-3′ 3′- GG↑CGCC-5′	40	20	<20	<20	**100**	–
SalI	5′-G↓TCGA C-3 3′-C AGCT↑G-5′	<20	<20	**100**	20*	<20	–
ScaI	5′-AGT↓ACT-3′ 3′-TCA↑TGA-5′	<20*	<20*	**100**	60*	<20*	–
SmaI	5′- CCC↓GGG-3′ 3′- GGG↑CCC-5′	<20	<20	<20	<20	**100**	–
SpeI	5′-A↓CTAGT-3′ 3′-TGATC↑A-5′	80*	**100**	80	100	80*	–
SphI	5′- GCATG↓C-3′ 3′- C↑GTACG-5′	20*	40*	**100**	120	20*	–
SspI	5′-AAT↓ATT-3′ 3′-TTA↑TAA-5′	<20*	60*	40	100*	80*	**100** (SSpI buffer)
StuI	5′-AGG↓CCT-3′ 3′-TCC↑GGA-5′	60	**100**	60	80	140	–
TaqI (TthHB8I)	5′- T↓CGA-3′ 3′- AGC↑T-5′	40*	80*	60*	60*	80*	**100** (TaqI buffer)
XbaI	5′- T↓CTAGA -3 3′- AGATC↑T-5′	<20	**80***	20	<20	120	–
XhoI (PaeR7I)	5′-C↓TCGAG-3′ 3′-GAGCT↑C-5′	<20	60	**100**	160	60	–

*Weak star activity is detected.
**100% activity is obtained by addition of 0.01% BSA.



Buffer compositions

10X Buffer H

500 mM Tris-HCl, pH 7.5

100 mM $MgCl_2$

10 mM dithiothreitol (DTT)

1000 mM NaCl

10X Buffer K

200 mM Tris-HCl, pH 8.5

100 mM $MgCl_2$

10 mM dithiothreitol (DTT)

1000 mM KCl

10X Buffer L

100 mM Tris-HCl, pH 7.5

100 mM $MgCl_2$

10 mM dithiothreitol (DTT)

10X Buffer M

100 mM Tris-HCl, pH 7.5

100 mM $MgCl_2$

10 mM dithiothreitol (DTT)

500 mM NaCl

10X Buffer T (BSA-free)

330 mM Tris-acetate, pH 7.9

100 mM magnesium acetate

5 mM dithiothreitol (DTT)

660 mM potassium acetate

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