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Not for use in diagnostic procedures.

Restriction Endonuclease Asp700 I(Xmn I)

From *Acinetobacter species* 700 (formerly *Achromobacter sp.*)

Cat. No. 10 835 277 001 500 units (10 U/µl)

GAANN↓NNTTC
CTTNN↑NNAAG

Version 19
Content version: February 2016
Store at −15 to −25°C

Stability/Storage The undiluted enzyme solution is stable when stored at −15 to −25°C until the control date printed on the label. Do not store below −25°C to avoid freezing.

Sequence specificity Asp700 I is an isoschizomer of Xmn I, recognizes the sequence GAANN/NNTTC and generates fragments

Isoschizomers The enzyme is an isoschizomer to MroX I, Pdm I and Xmn I.

Methylation sensitivity Asp700 I is inhibited by the presence of 6-methyladenine as indicated (*). The presence of the other A as 6-methyladenine or the presence of 5-methylcytosine is not inhibiting (°).

Storage buffer 20 mM Tris-HCl, 250 mM NaCl, 0.02 % polydocanol, 0.1 mM PMSF, 0.01 % gelatine, 0.1 mM EDTA, 10 mM 2-Mercaptoethanol, 50% Glycerol (v/v), pH approx. 8.5 (at 4°C).

Suppl. Incubation buffer, (10x) 100 mM Tris-HCl, 1 M NaCl, 50 mM $MgCl_2$, 10 mM 2-Mercaptoethanol, pH 8.0 (at 37°C), (= SuRE/Cut Buffer **B)**

Activity in SuRE/Cut Buffer System Bold face printed buffer indicates the recommended buffer for optimal activity:

A	**B**	L	M	H
50-75%	**100%**	10-25%	50-75%	0-10%

Incubation temperatur **37°C**

Unit definition One unit is the enzyme activity that completely cleaves 1 µg λDNA in 1 h at **37°C** in a total volume of 25 µl in SuRE/Cut buffer **B**

Typical experiment

Component	Final concentration
DNA	1 µg
10 × SuRE/Cut Buffer **B**	2.5 µl
Repurified water	Up to a total volume of 25 µl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

Heat inactivation The enzyme can be heat inactivated by heating to 65°C for 15 min.

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
24	5	0	3	2	2	1	1

Activity in PCR buffer Relative activity in PCR mix (Taq DNA Polymerase buffer) is **20%**. The PCR mix contained λ target DNA, primers, 10 mM Tris-HCl (pH 8.3, 20°C), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

Ligation and recutting assay Asp700 I fragments obtained by complete digestion of 1 µg λDNA are ligated with 1 U T4-DNA ligase in a volume of 10 µl by incubation for 16 h at 4°C in 66 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM ATP, pH 7.5 (at 20°C) resulting in >85 % recovery of 1 µg λDNA fragments. Subsequent re-cutting with Asp700 I yields > 95% of the typical pattern of λDNA × Asp700 I fragments.

Troubleshooting A critical component is the DNA substrate. Many compounds used in the isolation of DNA such as phenol, chloroform, ethanol, SDS, high levels of NaCl, metal ions (*e.g.*, Hg^{2+}, Mn^{2+}) inhibit or alter recognition specificity of many restriction enzymes. Such compounds should be removed by ethanol precipitation followed by drying, before the DNA is added to the restriction digest reaction. Appropriate mixing of the enzyme is recommended.

Star activity The Asp700 I sequence specificity is relaxed at low ionic strength or by addition of glycerol, ethanol or DMSO to the incubation mixture (3)

Quality control

Lot-specific certificates of analysis are available at www.lifescience.roche.com/certificates

Absence of unspecific endonuclease activities 1 µg λDNA is incubated for 16 h in 50 µl SuRE/Cut buffer B with excess of Asp700 I. The number of enzyme units which do not change the enzyme-specific pattern is stated in the certificate of analysis.

Absence of exonuclease activity Approx. 5 µg [^{3}H] labeled calf thymus DNA are incubated with 3 µl Asp700 I for 4 h at 37°C in a total volume of 100 µl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithioerythritol, pH approx. 7.5. Under these conditions, no release of radioactivity is detectable, as stated in the certificate of analysis.

References

1 Bolton, B. J. *et al.* (1985) *FEBS Letters* **182** (1), 130–134.
2 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1–248.
3 Rebase The Restriction Enzyme Database: http://rebase.neb.com
4 Benchmate: http://www.roche-applied-science.com/benchmate

Ordering Information

Product	Application	Packsize	Cat. No.
Rapid DNA Ligation Kit	Ligation of sticky- or blunt-ended DNA fragments in just 5 min at +15 to +25 °C.	Kit (40 DNA ligations)	11 635 379 001
T4 DNA Ligase	Ligation of sticky- and blunt- ended DNA fragments.	100 U 500 units (1 U/µl)	10 481 220 001 10 716 359 001
rAPid Phosphatase	Dephosphorylation of 5´-phosphate residues from nucleic acids	1000 U 5000 U	04 898 133 001 04 898 141 001
rAPid Dephos and Ligation Kit	Dephosphorylation of nucleic acids.	40 reactions 160 reactions	04 898 117 001 04 898 125 001
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5´-phosphate residues from nucleic acids.	1000 U (20 U/µl)	11 097 075 001
Agarose MP	Multipurpose agarose for analytical and preparative electrophoresis of nucleic acids	100 g 500 g	11 388 983 001 11 388 991 001
Agarose LE	Separation of nucleic acids in the range 0.2 - 1.5 kbp	100 g 500 g	11 685 660 001 11 685 678 001
Agarose Gel DNA Extraction Kit	For the elution of DNA fragments from agarose gels.	1 Kit (max. 100 reactions)	11 696 505 001
High Pure PCR Product Purification Kit	Purification of PCR or enzymatic modification reaction (*e.g.* restriction digest)	50 purifications 250 purifications	11 732 668 001 11 732 676 001
SuRE/Cut Buffer Set for Restriction Enzymes	Incubation buffers A, B, L, M and H for restriction enzymes	1 ml each (10× conc. solutions)	11 082 035 001
SuRE/Cut Buffer A	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 959 001
SuRE/Cut Buffer B	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 967 001
SuRE/Cut Buffer H	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 991 001
SuRE/Cut Buffer L	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 975 001
SuRE/Cut Buffer M	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 983 001
Water, PCR Grade	Specially purified, double-distilled, deionized, and autoclaved	100 ml (4 vials of 25 ml) 25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001
BSA, special quality for molecular biology	Maintaining enzyme stability	20 mg (1 ml)	10 711 454 001

Changes to previous version

Editorial changes

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Commonly used bacterial strains

Strain	Genotype
BL21	*E. coli B F$^-$ dcm ompT hsdS(r_B- m_B-) gal* (Studier, F.W. *et al* (1986) *J. Mol. Biol.*, **189,** 113.)
C600^e	*supE44 hsdR2 thi-1 thr-1 leuB6 lacY1 tonA21*; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
DH5α	*supE44* Δ(*lac*U169 (φ80d*lacZ*ΔM15) *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
HB101	*supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*; (Hanahan, D., (1983) *J. Mol. Biol.* **166**, 557.)
JM108	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB); (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33**, 103.)
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lac*I^q *lac*ZΔM15]; (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33**, 103.)
JM110	*rpsL (Str^r) thr leu thi-l lacY galK galT ara tonA tsx dam dcm supE44* Δ*(lac-proAB) F'[traD36proAB*$^+$ *, lacI^q lacZΔM15];* (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33**, 103.)
K802	*supE hsdR gal metB;* (Raleigh, E. et al., (1986) Proc.Natl. Acad.Sci USA, 83, 9070.; Wood, W.B. (1966) J. Mol. Biol., **16**, 118.)
SURE^r	*recB recJ sbc C201 uvrC umuC::Tn5(kan^r) lac* , Δ(hsdRMS) endA1 gyrA96 thi relA1 supE44 F'[proAB$^+$ lacI^q lacZΔM15 Tn10 (tet^r); (Greener, A. (1990) Stratagies, **3**, 5.)
TG1	*supE hsd Δ5 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lac*I^q *lac*ZΔM15]; (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue^r	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F'[*proAB*$^+$, *lac*I^q *lac*ZΔM15 Tn10 *(tet^r)*]; (Bullock *et al.,* (1987) BioTechniques, 5, 376.)

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