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EZ-Tn5™ <KAN-2>Tnp Transposome™ Kit

Cat. No. TSM99K2

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1. Introduction

The EZ-Tn5™ <KAN-2>Tnp Transposome™ is the stable complex formed between the EZ-Tn5 Transposase enzyme and the EZ-Tn5 <KAN-2> Transposon. The EZ-Tn5 <KAN-2> Transposon contains the Tn903 kanamycin resistance gene (Kan^R) that is functional in *E. coli*, flanked by hyperactive 19-basepair Mosaic End (ME) EZ-Tn5 Transposase recognition sequences. The EZ-Tn5 Transposome can be electroporated into living cells where the EZ-Tn5 Transposase is activated by Mg^{2+} in the host's cellular environment resulting in random insertion of the EZ-Tn5 Transposon into the host genomic DNA.[1-5]

Unlabeled forward and reverse transposon-specific primers are supplied in the kit. The primers can be used for bi-directional DNA sequencing or mapping of transposon insertion sites in target genomic DNAs, including direct sequencing from genomic DNA without cloning.[3]

2. Applications

- Create gene "knockouts" by insertional mutagenesis, *in vivo*.†
- Sequence bacterial genomic DNA directly from transposon insertions, **without** cloning.
- Insert priming sites for mapping or PCR into chromosomal DNA, *in vivo*.
- Insert kanamycin-resistance marker into bacteria (e.g., *E. coli*, *Salmonella typhimurium, Proteus vulgaris,* and others), *in vivo*.

3. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: The EZ-Tn5 <KAN-2>Tnp Transposome is supplied in a 50% glycerol solution containing 27.5 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.3 mM EDTA, 0.05% Triton®X-100, and 0.5 mM dithiothreitol. The KAN-2 FP-1 Forward and KAN-2 RP-1 Reverse Primers are supplied in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

Size: Reagents included in the kit are sufficient for 10 *in vivo* transposon insertion reactions.

Quality Control: EZ-Tn5 <KAN-2>Tnp Transposome activity is assayed by electroporation into a *rec*A$^-$ *E. coli* host strain having a transformation efficiency of $>10^9$ cfu/µg DNA. Assays must yield $>10^5$ Kan^R colonies/µg or $>2 \times 10^3$ Kan^R colonies/µl of transposome respectively. Primers are function-tested in a DNA cycle sequencing reaction using the SequiTherm EXCEL™ II DNA Sequencing Kit and in a PCR reaction using a plasmid containing an EZ-Tn5 <KAN-2> Transposon as template.

Contaminating Activity Assays: All components of the EZ-Tn5 <KAN-2>Tnp Transposome Kit are free of detectable DNase and RNase activities as judged by agarose gel electrophoresis following over-digestion assays, with the exception of the inherent endonucleolytic function of the EZ-Tn5 Transposase.

4. Kit Contents

Desc.	Concentration	Quantity
EZ-Tn5™ <KAN-2>Tnp Transposome™ Kit Contents		
EZ-Tn5™ <KAN-2>Tnp Transposome™	@ 20 ng/µl	10 µl
KAN-2 FP-1 Forward Primer	@ 50 mM	20 µl
KAN-2 RP-1 Reverse Primer	@ 50 mM	20 µl
Sterile Water		1 ml

5. Related Products

The following products are also available:

- TransforMax™ EC100™ Electrocompetent *E. coli*
- MasterPure™ DNA Purification Kits
- EZ-Tn5™ Tnp Transposome™ Kits
- EZ-Tn5™ Transposase
- EZ-Tn5™ Transposons
- EZ-Tn5™ Insertion Kits

6. Protocol

Electroporation of Host Cells with EZ-Tn5 <KAN-2>Tnp Transposome and Selection of Transposition Clones:

Electroporate electrocompetent cells using 1 µl of the EZ-Tn5 <KAN-2> Tnp Transposome. The electrocompetent cells should have a transformation efficiency of $>10^7$ cfu/µg of DNA, but use cells of the highest transformation efficiency possible to maximize the number of transposon insertion clones. Perform electroporation according to the equipment manufacturer's recommendations.

Immediately recover the electroporated cells after electroporation. Even slight delays in initiating the cell recovery process will result in a reduced number of transposition clones. For *E. coli*, add SOC medium to the electroporation cuvette to 1 ml final volume **immediately** after electroporation. Pipette the medium/cells gently to mix. Transfer to a tube and incubate on a 37°C shaker for 30-60 minutes to facilitate cell outgrowth.

If working with *E. coli*, dilute aliquots of the recovered cells (e.g., 1:10 and 1:100). Plate 100 µl of undiluted cells and each cell dilution separately on plates containing 50 µg/ml kanamycin. Other species may require plating of undiluted cells on plates containing 25-50 µg/ml kanamycin. Store the unused portion of the electroporated cells at +4°C for up to 2 days in the event that additional plates need to be prepared. The number of Kan^R colonies/µl of EZ-Tn5 <KAN-2>Tnp Transposome will be dependent on the transformation efficiency of the cells used and the level of expression of the Tn903 kanamycin resistance marker in that species. Epicentre's TransforMax EC100 Electrocompetent *E. coli* (available separately) have a transformation efficiency of $>1 \times 10^9$ cfu/µg and are ideal for this application.

Typical Kan^R colonies/µl of EZ-Tn5 <KAN-2>Tnp Transposome (20 ng) produced by electroporation:

Escherichia coli	*Salmonella typhyimurium*	*Proteus vulgaris*	*Pseudomonas species*
$>10^5$	$>10^4$	$>10^3$	$>10^2$

7. Primer Information

KAN-2 FP-1 Forward Primer

5′ - ACCTACAACAAAGCTCTCATCAACC - 3′

Length: 25 nucleotides

G+C content: 11

Molecular Weight: 7,484 daltons

Temperatures of Dissociation & Melting:

T_d: 68°C (nearest neighbor method)

T_m: 73°C (% G+C method)

T_m: 72°C ([2 (A+T) + 4 (G+C)] method)

T_m: 63°C ((81.5 + 16.6 (log [Na^+])) + ([41 (#G+C) - 500] / length) method) where [Na^+] = 0.1 M

KAN-2 RP-1 Reverse Primer

5′ - GCAATGTAACATCAGAGATTTTGAG - 3′

Length: 25 nucleotides

G+C content: 9

Molecular Weight: 7,705 daltons

Temperatures of Dissociation & Melting:

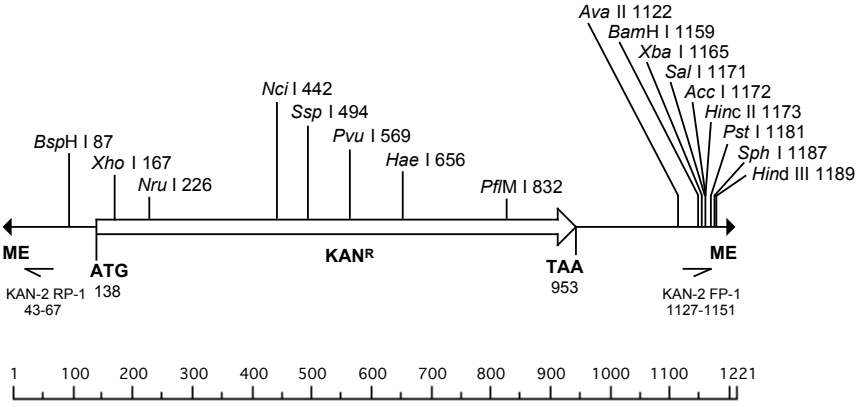
T_d: 65°C (nearest neighbor method)

T_m: 69°C (% G+C method)

T_m: 68°C ([2 (A+T) + 4 (G+C)] method)

T_m: 60°C ((81.5 + 16.6 (log [Na^+])) + ([41 (#G+C) - 500] / length) method) where [Na^+] = 0.1 M

EZ-Tn5™ <KAN-2> Transposon
(1221 bp.)



Note: Not all restriction enzymes that cut only once are indicated above.
See the following pages for further information.
Primers are not drawn to scale.

KAN-2 FP-1 Forward Primer	5' ACCTACAACAAAGCTCTCATCAACC 3'
KAN-2 RP-1 Reverse Primer	5' GCAATGTAACATCAGAGATTTTGAG 3'
ME = Mosaic End	5' AGATGTGTATAAGAGACAG 3'

Figure 1. EZ-TN5 <KAN-2> Transposon.

Restriction Enzymes that cut the EZ-Tn5 <KAN-2> Transposon one to three times:

Enzyme	Sites	Location	Enzyme	Sites	Location
Acc I	1	1172	Fau I	1	1148
Aci I	1	174	Fnu4H I	2	174, 1013
Alu I	2	1139, 1191	Hae I	1	656
Apo I	2	182, 366	Hae III	2	173, 656
AsiS I	1	569	Hinc II	1	1173
Ase I	1	768	Hind III	1	1189
Ava I	1	167	Hpa II	3	442, 524, 705
Ava II	1	1122	HpyCH4 IV	1	159
BamH I	1	1159	Mae II	1	159
Ban II	1	224	Mbo II	3	369, 480, 1058
Bfa I	1	1166	Mly I	2	802, 1178
BfrB I	2	417, 683	Msp I	3	443, 525, 706
BfuA I	1	1184	Mwo I	3	281, 313, 527
Bpu10 I	1	586	Nci I	1	442
BsaW I	1	704	Nla IV	1	1161
BsiE I	1	569	Nru I	1	226
Bsm I	2	453, 530	Nsi I	2	419, 685
BsmB I	1	585	Nsp I	1	1187
Bsp1286 I	1	224	PaeR7 I	1	167
BspD I	2	29, 260	PflM I	1	832
BspH I	1	87	Ple I	2	801, 1177
BspM I	1	1184	PspG I	2	457, 814
Bsr I	3	360, 984, 1124	Pst I	1	1181
BsrD I	1	61	Pvu I	1	569
BsrF I	1	523	Rsa I	1	404
BssK I	3	440, 457, 814	Sal I	1	1171
BstDS I	2	1093, 1154	Sau96 I	1	1122
BstF5 I	2	200, 826	Sbf I	1	1181
BstN I	2	459, 816	ScrF I	3	442, 459, 816
BstU I	3	176, 226, 571	Sfc I	1	1177
BstY I	2	818, 1159	Sml I	1	167
Btg I	2	1089, 1150	Sph I	1	1187
Bts I	2	430, 517	Ssp I	1	494
Cla I	2	29, 260	Tli I	1	167
Dde I	2	586, 1041	Tsp45 I	1	716
Dsa I	2	1089, 1150	TspR I	3	442, 517, 989
Ear I	1	382	Xba I	1	1165
EcoN I	1	481	Xho I	1	167

Restriction Enzymes that cut the EZ-Tn5 <KAN-2> Transposon four or more times:

Alw I	CviJ I	Hph I	Mbo I	SfaN I
BsaJ I	Dpn I	Hpy188 I	Mnl I	Taq I
Bsl I	Hha I	HpyCH4 III	Mse I	Tfi I
BsmA I	Hinf I	HpyCH4 V	Nla III	Tsp4C I
Cac8 I	HinP I	Mae III	Sau3A I	Tsp509 I

Restriction Enzymes that do not cut the EZ-Tn5 <KAN-2> Transposon:

Aat II	Blp I	Bsu36 I	Nae I	Sca I
Acc65 I	Bme1580 I	Dra I	Nar I	SexA I
Acl I	BmgB I	Dra III	Nco I	Sfi I
Afe I	Bmr I	Drd I	Nde I	Sfo I
Afl II	Bsa I	Eae I	NgoM IV	SgrA I
Afl III	BsaA I	Eag I	Nhe I	Sim I
Age I	BsaB I	Eco47 III	Not I	Sma I
Ahd I	BsaH I	EcoO109 I	Pac I	SnaB I
Ale I	BseY I	*Eco*R I	Pci I	Spe I
AlwN I	BsiHKA I	*Eco*R V	PflF I	Srf I
Apa I	BsiW I	Fse I	Pme I	Sse8647 I
ApaB I	BspE I	Fsp I	Pml I	Stu I
ApaL I	BspLU11 I	Gdi II	PpuM I	Sty I
Asc I	BsrB I	Hae II	PshA I	Swa I
Avr II	BsrG I	Hpa I	Psi I	Tat I
Ban I	BssH II	Hpy99 I	PspOM I	Tse I
Bbs I	BssS I	Kpn I	Pvu II	Tth111 I
BbvC I	BstAP I	Mfe I	Rsr II	Xcm I
BciV I	BstB I	Mlu I	Sac I	Xma I
Bcl I	BstE II	Msc I	Sac II	Xmn I
Bgl I	BstX I	Msl I	SanD I	
Bgl II	BstZ17 I	MspA1 I	Sap I	

EZ-Tn5 <KAN-2> Transposon Sequence

EZ-Tn5™ <KAN-2> Transposon 1,221 bp.

1	CTGTCTCTTA	TACACATCTC	AACCATCATC	GATGAATTGT	GTCTCAAAAT
51	CTCTGATGTT	ACATTGCACA	AGATAAAAAT	ATATCATCAT	GAACAATAAA
101	ACTGTCTGCT	TACATAAACA	GTAATACAAG	GGGTGTTATG	AGCCATATTC
151	AACGGGAAAC	GTCTTGCTCG	AGGCCGCGAT	TAAATTCCAA	CATGGATGCT
201	GATTTATATG	GGTATAAATG	GGCTCGCGAT	AATGTCGGGC	AATCAGGTGC
251	GACAATCTAT	CGATTGTATG	GGAAGCCCGA	TGCGCCAGAG	TTGTTTCTGA
301	AACATGGCAA	AGGTAGCGTT	GCCAATGATG	TTACAGATGA	GATGGTCAGA
351	CTAAACTGGC	TGACGGAATT	TATGCCTCTT	CCGACCATCA	AGCATTTTAT
401	CCGTACTCCT	GATGATGCAT	GGTTACTCAC	CACTGCGATC	CCCGGAAAAA
451	CAGCATTCCA	GGTATTAGAA	GAATATCCTG	ATTCAGGTGA	AAATATTGTT
501	GATGCGCTGG	CAGTGTTCCT	GCGCCGGTTG	CATTCGATTC	CTGTTTGTAA
551	TTGTCCTTTT	AACAGCGATC	GCGTATTTCG	TCTCGCTCAG	GCGCAATCAC
601	GAATGAATAA	CGGTTTGGTT	GATGCGAGTG	ATTTTGATGA	CGAGCGTAAT
651	GGCTGGCCTG	TTGAACAAGT	CTGGAAAGAA	ATGCATAAAC	TTTTGCCATT
701	CTCACCGGAT	TCAGTCGTCA	CTCATGGTGA	TTTCTCACTT	GATAACCTTA
751	TTTTTGACGA	GGGGAAATTA	ATAGGTTGTA	TTGATGTTGG	ACGAGTCGGA
801	ATCGCAGACC	GATACCAGGA	TCTTGCCATC	CTATGGAACT	GCCTCGGTGA
851	GTTTTCTCCT	TCATTACAGA	AACGGCTTTT	TCAAAAATAT	GGTATTGATA
901	ATCCTGATAT	GAATAAATTG	CAGTTTCATT	TGATGCTCGA	TGAGTTTTTC
951	TAATCAGAAT	TGGTTAATTG	GTTGTAACAC	TGGCAGAGCA	TTACGCTGAC
1001	TTGACGGGAC	GGCGGCTTTG	TTGAATAAAT	CGAACTTTTG	CTGAGTTGAA
1051	GGATCAGATC	ACGCATCTTC	CCGACAACGC	AGACCGTTCC	GTGGCAAAGC
1101	AAAAGTTCAA	AATCACCAAC	TGGTCCACCT	ACAACAAAGC	TCTCATCAAC
1151	CGTGGCGGGG	ATCCTCTAGA	GTCGACCTGC	AGGCATGCAA	GCTTCAGGGT
1201	TGAGATGTGT	ATAAGAGACA	G		

The transposon sequence can be downloaded at the URL: http://www.epicentre.com/sequences

8. References

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