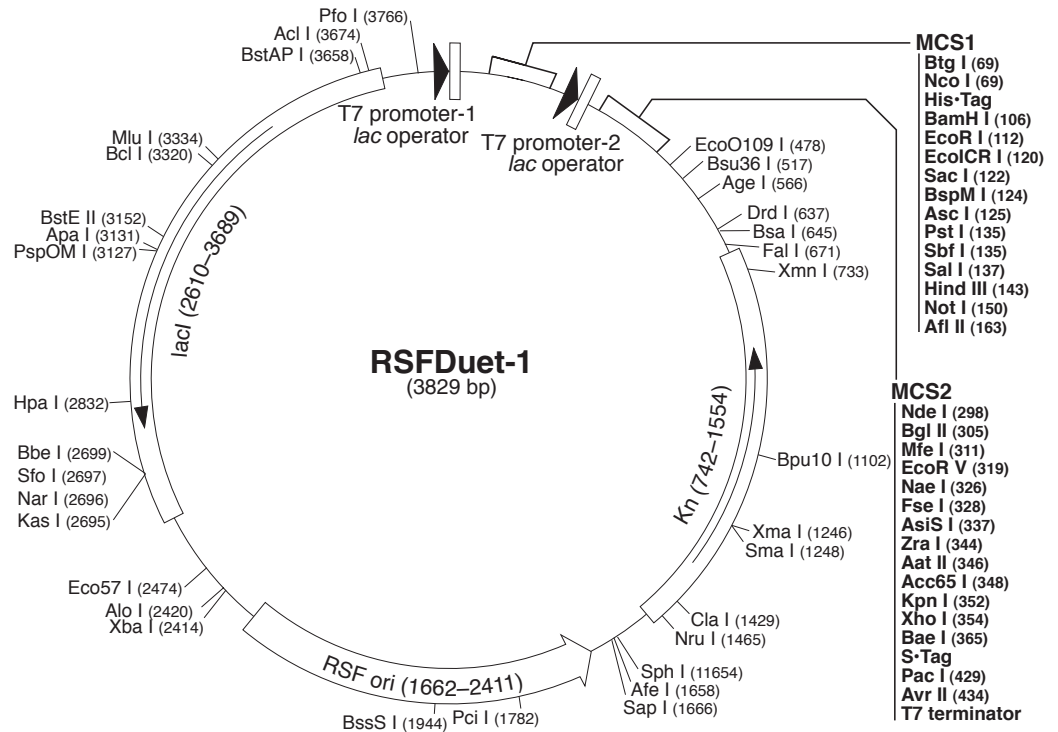


pRSFDuet-1 Vector

	Cat. No.
pRSFDuet-1 DNA	71341-3
pRSFDuet-1 sequence landmarks	
T7 promoter-1	3582-3598
T7 transcription start-1	1
His•Tag [®] coding sequence	83-100
Multiple cloning sites-1 (<i>Nco</i> I- <i>Afl</i> II)	69-168
T7 promoter-2	214-230
T7 transcription start-2	231
Multiple cloning sites-2 (<i>Nde</i> I- <i>Avr</i> II)	297-438
S•Tag [™] coding sequence	366-410
T7 terminator	462-509
kan (<i>Kn^R</i>) coding sequence	742-1554
RSF origin	1662-2411
<i>lacI</i> coding sequence	2610-3689

pRSFDuet[™]-1 is designed for the coexpression of two target ORFs. The vector contains two multiple cloning sites (MCS), each of which is preceded by a T7*lac* promoter and ribosome binding site (rbs). The vector also carries the RSF1030-derived RSF replicon, *lacI* gene and kanamycin resistance gene (*Kn^R*). This vector can be used in combination with pACYCDuet[™]-1 (Cat. No. 71147-3), pCDFDuet[™]-1 (Cat. No. 71340-3), and pETDuet[™]-1 (Cat. No. 71146-3) in an appropriate host strain for the coexpression of 4 to 8 target proteins. ORFs inserted into MCS1 can be sequenced using the ACYCDuetUP1 Primer (Cat. No. 71178-3) and DuetDOWN1 Primer (Cat. No. 71179-3). ORFs inserted into MCS2 can be sequenced using the DuetUP2 Primer (Cat. No. 71180-3) and T7 Terminator Primer (Cat. No. 69337-3). Unique sites are shown on the circle map.



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                    Pfo I (3766)
                    Acl I (3674)
                    BstAP I (3658)
                    T7 promoter-1
                    lac operator
                    T7 promoter-2
                    lac operator
                    EcoO109 I (478)
                    Bsu36 I (517)
                    Age I (566)
                    Drd I (637)
                    Bsa I (645)
                    Fal I (671)
                    Xmn I (733)
                    MCS1
                    Btg I (69)
                    Nco I (69)
                    His-Tag
                    BamH I (106)
                    EcoR I (112)
                    EcoCR I (120)
                    Sac I (122)
                    BspM I (124)
                    Asc I (125)
                    Pst I (135)
                    Sbf I (135)
                    Sal I (137)
                    Hind III (143)
                    Not I (150)
                    Afl II (163)
                    MCS2
                    Nde I (298)
                    Bgl II (305)
                    Mfe I (311)
                    EcoR V (319)
                    Nae I (326)
                    Fse I (328)
                    AsiS I (337)
                    Zra I (344)
                    Aat II (346)
                    Acc65 I (348)
                    Kpn I (352)
                    Xho I (354)
                    Bae I (365)
                    S-Tag
                    Pac I (429)
                    Avr II (434)
                    T7 terminator
                    Pfu I (3766)
                    Acl I (3674)
                    BstAP I (3658)
                    Mlu I (3334)
                    Bcl I (3320)
                    BstE II (3152)
                    Apa I (3131)
                    PspOM I (3127)
                    Hpa I (2832)
                    Bbe I (2699)
                    Sfo I (2697)
                    Nar I (2696)
                    Kas I (2695)
                    Eco57 I (2474)
                    Ajo I (2420)
                    Xba I (2414)
                    BSS S I (1944)
                    Pci I (1782)
                    Sph I (11654)
                    Afe I (1658)
                    Sap I (1666)
                    Cla I (1429)
                    Nru I (1465)
                    Xma I (1246)
                    Sma I (1248)
                    Bpu10 I (1102)
                    Kn (742-1554)
                    RSF ori (1662-2411)
                    lacI (2610-3689)
                    RSFDuet-1 (3829 bp)
    
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                    ACYCDuetUP1
                    Primer #71178-3
                    T7 promoter-1
    GCCATACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGGACTCCTGCATTAGGAAATTAATACGACTCACTATA
    T7 transcription start-1
    lac operator
    rbs
    Nco I
    His-Tag
    GGGGAATTGTGAGCGGATAACAATCCCTCGTAGAATAATTTGTTTAACTTAATAAGGAGATATACCATGGGCAGCAGCCATCACCATCATCACCAC
    MetGlySerSerHisHisHisHisHisHis
    BspM I
    BamH I
    EcoCR I
    Sac I
    Asc I
    Sbf I
    Pst I
    Sal I
    Hind III
    Not I
    Afl II
    Tat I
    DuetUP2 Primer
    BsrG I
    #71180-3
    AGCCAGGATCCGAATTCGAGCTCGGCGCCCTGCAGGTCGACAAGCTTGCAGCCGATAATGCTTAAGTCGAACAGAAAGTAATCGTATTTGTACACGGCC
    SerGlnAspProAsnSerSerSerAlaArgLeuGlnValAspLysLeuAlaAlaAlaEnd
    DuetUP1 Primer
    #71180-3
    T7 promoter-2
    T7 transcription start-2
    lac operator
    rbs
    Nde I
    GCATAATCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCCATCTTAGTATATTAGTTAAGTATAAGGAGAGATATACAT
    DuetDOWN1 Primer
    #71179-3
    Nde I
    Bgl II
    Mfe I
    EcoR V
    Fse I
    Pvu I
    Zra I
    Kpn I
    Bae I
    S-Tag
    ATGGCAGATCTCAATTGGATATCGGCGGCCACGCGATCGCTGACGCTGGTACCTCGAGTCTGGTAAAGAAACCGCTGCTGCGAAATTTGAACGCCAG
    MetAlaAspLeuAsnTrpIleSerAlaGlyHisAlaIleAlaAspValGlyThrLeuGluSerGlyLysGluThrAlaAlaAlaLysPheGluArgGln
    S-Tag
    Pac I
    Avr II
    EcoO109 I
    CACATGGACTCGTCTACTAGCGCAGCTTAATTAACCTAGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTG
    HisMetAspSerSerThrSerAlaAlaEnd
    T7 Terminator Primer
    #69337-3
    
```

pRSFDuet-1 cloning/expression regions

pRSFDuet-1 Restriction Sites

TB391 0903

Enzyme	# Sites	Locations
AatII	1	346
Acc65I	1	348
AccI	2	138 411
AccII	1	3674
AfeI	1	1658
AflIII	1	163
AflIII	2	1782 3334
AgeI	1	566
Alol	1	2420
ApaI	1	3131
ApaLI	2	2085 3354
AscI	1	125
Asel	6	213 732 921 2592 2651 3812
AsiSI	1	337
AvaI	2	354 1246
AvrII	1	433
BaeI	1	365
BamHI	1	106
BanI	4	348 2565 2695 3414
BanII	3	122 1471 3131
BbeI	1	2699
BbsI	2	2849 3188
BceAI	4	211 801 2850 3477
BcgI	2	162 3014
BciVI	4	728 1604 1974 2882
BclI	1	3320
BfrBI	2	1008 1274
BglII	1	305
BlpI	2	451 2309
Brne1580I	3	2089 3131 3358
Bmrl	3	2536 3176 3413
Bpml	2	3013 3502
Bpu10I	1	1102
BpuEI	5	515 1862 2160 2340 2526
BsaHI	3	343 2696 3379
BsaI	1	645
BsaWI	8	551 566 983 1804 1977 2124 2512 3015
BsaXI	2	655 2666
BseYI	3	2075 2800 2935
BsgI	2	3289 3489
BsiEI	9	153 199 325 337 636 1124 1698 2111 2555
BsiHKAII	3	122 2089 3358
BsmAI	7	645 1102 1604 2719 3106 3232 3637
BsmBI	2	1102 2719
BsmI	2	1163 1240
Bsp1286I	5	122 1471 2089 3131 3358
BspCNI	6	443 530 1094 2059 2322 2755
BspHI	2	725 1602
BspLU111	1	1782
BspMI	1	124
BsrBI	5	13 243 723 1608 1715
BsrDI	2	2927 3293
BsrFI	4	324 566 1164 3648
BsrGI	1	190
BssHII	2	125 2923
BssSI	1	1944
BstAPI	1	3658
BstEII	1	3152
BstXI	3	3288 3411 3540
BstYI	5	106 305 869 2558 3770
Bsu36I	1	517
BtgI	1	69
BtsI	5	543 1176 1263 2607 2975
Clal	1	1429
DrdI	1	637
EaeI	5	150 196 322 326 2660
EagI	3	150 196 322

Enzyme	# Sites	Locations
EarI	5	1306 1562 1666 2442 3717
EciI	3	1833 1979 3549
Ecl136II	1	120
Eco57I	1	2474
Eco57MI	3	2474 3013 3502
EcoCRI	1	120
EcoNI	2	1209 3802
EcoO109I	1	478
EcoRI	1	112
EcoRV	1	319
FalI	1	671
FseI	1	328
HaeII	5	1660 2019 2699 2942 3723
HincII	3	139 2392 2832
HindIII	1	143
HpaI	1	2832
KasI	1	2695
KpnI	1	352
MfeI	1	311
MluI	1	3334
MsiI	3	2968 2998 3286
NaeI	1	326
NarI	1	2696
NcoI	1	69
NdeI	1	298
NgoMIV	1	324
NottI	1	150
NruI	1	1465
NsiI	2	1010 1276
NspI	2	1654 1786
PaclI	1	429
PciI	1	1782
PfIMI	3	401 862 3759
PfoI	1	3766
PinAI	1	566
PspOMI	1	3127
PstI	1	135
PvuI	1	337
PvuII	2	2645 2738
SacI	1	122
SalI	1	137
SapI	1	1666
SbfI	1	135
SfciI	4	29 131 226 3825
SfoI	1	2697
SmaI	1	1248
SmlI	7	163 354 494 1877 2139 2319 2541
SphI	1	1654
Sse8387I	1	135
SspI	2	1197 1571
StyI	4	69 433 473 2287
TaqII	4	870 1684 2368 2541
TatI	1	190
TspGWI	3	1303 1315 2396
Tth111I	2	637 2209
XbaI	1	2414
XcmI	3	2949 2967 3483
XhoI	1	354
XmaI	1	1246
XmnI	1	733
ZraI	1	344

Enzymes that do not cut pRSFDuet-1:

AarI	AhdI	AleI	AlwNI	BbvCI
BglI	BmgBI	BmtI	BpII	BsaAI
BsaBI	BseRI	BsiWI	BsmFI	BspEI
Bst1107I	BstBI	BstZ17I	BtrI	DraI
DraIII	FspAI	FspI	MscI	NheI
NspV	PmeI	PmlI	PpII	PpuMI
PshAI	Psil	Psrl	RsrII	SacII
SanDI	Scal	SexAI	Sfil	SgrAI
SnaBI	SpeI	SrlI	StuI	Swal