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## pCMV-N-Flag

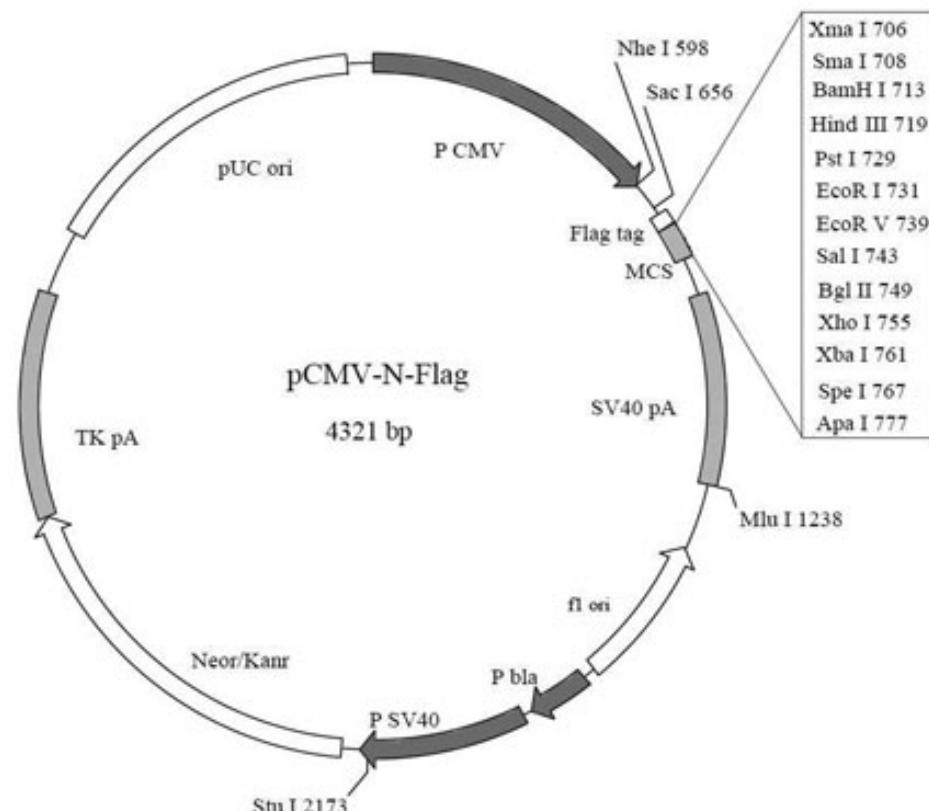
产品编号	产品名称	包装
D2722-1μg	pCMV-N-Flag	1μg
D2722-100μg	pCMV-N-Flag	100μg

### 产品简介:

- pCMV-N-Flag是碧云天自行研发的用于在哺乳动物细胞中表达N端和Flag tag (Flag标签)融合的目的蛋白的表达质粒。含有CMV启动子可以高效启动目的蛋白在细胞中的表达；在多克隆位点的5'端含有一个可以编码Flag标签的序列，因此可以表达出含有Flag标签的融合蛋白，可以方便地使用抗Flag的抗体来识别目的蛋白，有利于目的蛋白检测和分离纯化。质粒为卡那霉素抗性。转染细胞后，可使用G418筛选稳定表达目的蛋白的细胞株。
- pCMV-N-Flag质粒的主要信息如下：

Feature	Nucleotide	Position
CMV promoter		1-602
T3 promoter and T3 primer binding site		620-639
Flag tag		679-702
multiple cloning site		705-778
T7 promoter and T7 primer binding site		821-842
SV40 polyA signal		854-1237
f1 origin of ss-DNA replication		1375-1681
bla promoter		1706-1830
SV40 promoter		1850-2188
neomycin/kanamycin resistance ORF		2223-3014
HSV-thymidine kinase (TK) polyA signal		3015-3473
pUC origin		3602-4269

- pCMV-N-Flag质粒的图谱如下：



➤ pCMV-N-Flag的多克隆位点的详细图谱如下:

	Flag tag									
	M	D	Y	K	D	D	D	D	K	
SacI										
651	GAGCTCCACC	GCGGTGGCGG	CCGCCATGG	TTACAAGGAT	GACGACGATA					
	CTCGAGGTGG	CGCCACCGCC	GGCGGTACCT	AATGTTCTA	CTGCTGCTAT					
XmaI		PstI								
SmaI	BamHI	HindIII	EcoRI	EcoRV	SalI	BglII				
701	AGAGCCCCGGG	CGGATCCAAG	CTTCTGCAGG	AATTCGATAT	CGTCGACAGA					
	TCTCGGGGCC	GCCTAGGTTC	GAAGACGTCC	TTAAGCTATA	GCAGCTGTCT					
XhoI	XbaI		ApaI							
751	TCTCTCGAGT	CTAGAACTAG	TGGGCCCGGT	ACCTTAATTAA	ATTAAGGTAC					
	AGAGAGCTCA	GATCTTGATC	ACCCGGGCCA	TGGAATTAAAT	TAATTCCATG					

➤ pCMV-N-Flag中没有的酶切位点(Restriction enzymes that do not cut pCMV-N-Flag)包括:

Afl II	Age I	Ahd I	Asc I	Bbs I	Bbv II	Blp I
Bsg I	BsiW I	BsmB I	BspM II	BsrG I	BssH II	Bst1107 I
BstE II	Ear I	Eco47 III	Eco72 I	EcoN I	Esp I	Fse I
Nru I	PfLM I	Pme I	Pml I	PpuM I	Psp1406 I	Sap I
Sca I	Spl I					

➤ pCMV-N-Flag中的单酶切位点(Restriction enzymes that cut pCMV-N-Flag once)包括:

Nde I	CA`TA, TG	241	Pvu I	CG, AT`CG	855
SnaB I	TAC GTA	347	Bcl I	T`GATC, A	1009
Nhe I	G`CTAG, C	598	Mun I	C`AATT, G	1102
Sac I	G, AGCT`C	656	Hpa I	GTT AAC	1115
Sac II	CC, GC`GG	663	Mlu I	A`CGCG, T	1238
BstX I	CCAN, NNNN`NTGG	664	Dra III	CAC, NNN`GTG	1468
Not I	GC`GGCC, GC	669	Sfi I	GGCCN, NNN`NGGCC	2127
PspA I	C`CCGG, G	706	BseR I	GAGGAG 16/14	2170
Xma I	C`CCGG, G	706	Stu I	AGG CCT	2173
Srf I	GCCC GGGC	708	Cla I	AT`CG, AT	2192
Sma I	CCC GGG	708	Kas I	G`GCGC, C	2351
BamH I	G`GATC, C	713	Nar I	GG`CG, CC	2352
Hind III	A`AGCT, T	719	Ehe I	GGC GCC	2353
Pst I	C, TGCA`G	729	Bbe I	G, GCGC`C	2355
EcoR I	G`AATT, C	731	Msc I	TGG CCA	2434
EcoR V	GAT ATC	739	Tth111 I	GACN`N, NGTC	2470
Sal I	G`TCGA, C	743	BsrD I	GCAATG, 8	2585
Acc I	GT`MK, AC	744	Bsp1286 I	G, DGCH`C	2655
Bgl II	A`GATC, T	749	Rsr II	CG`GWC, CG	2868
PaeR7 I	C`TCGA, G	755	BsiC I	TT`CG, AA	3034
Xho I	C`TCGA, G	755	BstB I	TT`CG, AA	3034
Xba I	T`CTAG, A	761	Bsa I	GGTCTC 7/11	3341
Spe I	A`CTAG, T	767	HgiE II	ACCNNNNNNGGT-1/13	3681
Bsp120 I	G`GGCC, C	773	ApaL I	G`TGCA, C	3956
Apa I	G, GGCC`C	777			

➤ pCMV-N-Flag质粒中对于插入片段进行测序时，推荐使用的正向测序引物T3和反向测序引物T7的序列如下:

T3 primer (620-639): 5' AATTAACCTCTACTAAAGGG 3'  
 T7 primer (821-842): 5' GTAATACGACTCACTATAGGGC 3'

➤ pCMV-N-Flag的全序列信息请参考碧云天网站上该质粒的信息。

#### 包装清单:

产品编号	产品名称	包装
D2722-1μg	pCMV-N-Flag	1μg
D2722-100μg	pCMV-N-Flag	100μg
—	说明书	1份

#### 保存条件:

-20°C保存。

## 注意事项：

- 本质粒未经碧云天书面许可不得用于任何商业用途，也不得移交给订货人所在实验室外的任何个人或单位。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

## 使用说明：

1. 首次使用1μg包装的本产品时，请先取少量本质粒转化大肠杆菌，进行质粒小量、中量或大量抽提后再用于后续用途。抽提获得的质粒可以通过酶切电泳进行鉴定，或通过测序进行鉴定。
2. 100μg包装的本产品质粒浓度为0.1μg/μl，共1ml。可以直接用于酶切或者转染细胞。
3. pCMV-N-Flag质粒在其多克隆位点适当酶切后可以插入待表达的目的基因，需注意插入基因片段和tag之间的读码框要一致，即需要避免发生移码突变。构建的质粒可以用常规方法转染细胞。

## 使用本产品的文献：

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