

pLenti-BRCA1-sgRNA

产品编号	产品名称	包装
L00151	pLenti-BRCA1-sgRNA	5μg

产品简介:

- pLenti-BRCA1-sgRNA (BRCA1基因敲除质粒)是一种在动物细胞中可以同时表达Cas9、目的基因的sgRNA和puromycin抗性基因的质粒。用于在动物细胞中直接基于CRISPR/Cas9技术敲除目的基因，或者通过包装慢病毒后基于CRISPR/Cas9技术敲除目的基因。本质粒中sgRNA的有效性已经通过T7E1法的验证。
- 本质粒在细菌中为Amp抗性，全长约13,000bp。本质粒的关键图谱信息请参考图1。本质粒可直接转染细胞用于目的基因的CRISPR/Cas9敲除，以及通过puromycin筛选稳定细胞株；也可以与pMDLg、Rev及VSV-g共转HEK293T细胞进行重组慢病毒(lentivirus)的包装，然后再用于感染细胞或组织并进行目的基因的CRISPR/Cas9敲除。



图1. 表达sgRNA、Cas9和puromycin抗性的pLenti-sgRNA质粒关键图谱信息。

- 本质粒中的sgRNA基于碧云天研发的CRISPR/Cas9 sgRNA快速筛选和验证体系获得，sgRNA的有效性已经通过T7E1法验证。
- 本质粒用于实验时，建议同时选购无任何靶向的对照质粒pLenti-Control-sgRNA (L00011)或靶向GFP的对照质粒pLenti-GFP-sgRNA (L00013)。
- 碧云天同时提供基于CRISPR/Cas9技术的BRCA1基因敲除的质粒(L00151 pLenti-BRCA1-sgRNA)、慢病毒(L00152 BRCA1 Knockout Lentivirus)、HEK293T细胞(L00153 BRCA1 Knockout HEK293T Cells)、HEK293T敲除细胞的RIPA裂解液(L00154 BRCA1 Knockout HEK293T RIPA Lysate)、HEK293T敲除细胞的Trizol裂解液(L00155 BRCA1 Knockout HEK293T Trizol Lysate)等产品，具体请在碧云天网站查询或在本产品网点击相应产品。
- BRCA1基因的基本信息如下：

Species	Gene Symbol	Gene ID	GenBank Accession	Transcript
Human	BRCA1	672	BC072418	NM_007294

About the gene	
Official Symbol	BRCA1
Previous Symbol	-
Official Full Name	BRCA1, DNA repair associated
Synonyms	RNF53; BRCC1; PPP1R53; FANCS
Location	17q21.31
Gene Type	protein-coding gene
Uniprot ID	P38398
Pathway/Library	cAMP & Ca Signaling Pathway Related Genes Library; Ubiquitin Ligases Genes Library
Gene Summary	This gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumor suppressor. The encoded protein combines with other tumor suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex (BASC). This gene product associates with RNA polymerase II, and through the C-terminal domain, also interacts with histone deacetylase complexes. This protein thus plays a role in transcription, DNA repair of double-stranded breaks, and recombination. Mutations in this gene are responsible for approximately 40% of inherited breast cancers and more than 80% of inherited breast and ovarian cancers. Alternative splicing plays a role in modulating the subcellular localization and physiological function of this gene. Many alternatively spliced transcript variants, some of which are disease-associated mutations, have been described for this gene, but the full-length natures of only some of these variants has been described. A related pseudogene, which is also located on chromosome 17, has been identified.

<https://www.beyotime.com/product/ST551-10mg.htm>。

- c. 转染后约48小时，按照上述检测获得的puromycin筛选浓度加入puromycin，筛选阳性细胞。一般筛选2天后，阴性细胞逐渐死去。培养过程中，可以将细胞转至6孔板或10cm培养皿进行扩大培养。一周之后，puromycin浓度可减半。如果有必要后续可以通过将细胞稀释至2.5个/ml，然后按照每孔200 μ l接种到96孔板中(每孔平均0.5个细胞)，筛选单克隆细胞株。

5. 基因编辑的鉴定:

- a. 对于多克隆细胞，可以通过T7 Endonuclease I (T7EI)进行鉴定，即提取细胞的基因组DNA，在sgRNA序列两边设计引物进行PCR扩增，然后进行T7EI酶切，具体请参考碧云天的T7 Endonuclease I (CRISPR等基因突变鉴定用) (D7080)或基因组编辑突变检测试剂盒(D0508); 也可以通过相应的抗体进行检测。
- b. 对于单克隆细胞，可通过PCR扩增出sgRNA靶向的基因片段后进行常规测序的方式进行验证，同时也可以使用相应的抗体进行检测。

相关产品:

产品编号	产品名称	包装
L00002-5 μ g	CRISPR/Cas9 Packaging Vectors Set A	5 μ g/each
L00002-100 μ g	CRISPR/Cas9 Packaging Vectors Set A	100 μ g/each
L00011-5 μ g	pLenti-Control-sgRNA	5 μ g
L00011-100 μ g	pLenti-Control-sgRNA	100 μ g
L00013-5 μ g	pLenti-GFP-sgRNA	5 μ g
L00013-100 μ g	pLenti-GFP-sgRNA	100 μ g
C0222	青霉素-链霉素溶液(100X)	100ml
C0351-1ml	Polybrene (Hexadimethrine Bromide)	1ml
C0351-50mg	Polybrene (Hexadimethrine Bromide)	50mg
C0521	Lipo293 TM 转染试剂	0.5/1.5/7.5ml
C0526	Lipo6000 TM 转染试剂	0.5/1.5/7.5ml
C0533	Lipo8000 TM 转染试剂	0.5/1.5/7.5ml
D0378	Stbl3甘油菌	200 μ l
ST551-10mg	Puromycin Dihydrochloride (嘌呤霉素)	10mg/ml \times 1ml
ST551-50mg	Puromycin Dihydrochloride (嘌呤霉素)	10mg/ml \times 5ml
ST551-250mg	Puromycin Dihydrochloride (嘌呤霉素)	250mg
ST1380-500mg	Polybrene (\geq 94%, Reagent grade)	500mg
ST1380-2g	Polybrene (\geq 94%, Reagent grade)	2g
ST1380-10g	Polybrene (\geq 94%, Reagent grade)	10g
FF345-10pcs	针头滤器(0.45 μ m/28mm, PES, Sterile, Sartorius分装)	10个/袋
FF345T-10pcs	针头滤器(0.45 μ m/28mm, PES, Sterile, 进口分装)	10个/袋
FF345-50pcs	针头滤器(0.45 μ m/28mm, PES, Sterile, Sartorius原装)	50个/盒
FF365-10pcs	BeyoGold TM 针头滤器(0.45 μ m/33mm, PES, Sterile)	10个/袋
FF365-100pcs	BeyoGold TM 针头滤器(0.45 μ m/33mm, PES, Sterile)	100个/盒
FF375-10pcs	BeyoGold TM 针头滤器(0.45 μ m/13mm, PES, Sterile)	10个/袋
FF375-100pcs	BeyoGold TM 针头滤器(0.45 μ m/13mm, PES, Sterile)	100个/盒
FUF158-2pcs	超滤管(15ml, 100kDa MWCO, PES, Sartorius分装)	2个/袋
FUF158-12pcs	超滤管(15ml, 100kDa MWCO, PES, Sartorius分装)	12个/袋

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