

pLenti-DCLK1-sgRNA

产品编号	产品名称	包装
L15430	pLenti-DCLK1-sgRNA	5μg

产品简介:

- pLenti-DCLK1-sgRNA (DCLK1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DCLK1基因敲除的质粒(L15430 pLenti-DCLK1-sgRNA)、慢病毒(L15431 DCLK1 Knockout Lentivirus)、HEK293T细胞(L15432 DCLK1 Knockout HEK293T Cells)、HEK293T敲除细胞的RIPA裂解液(L15433 DCLK1 Knockout HEK293T RIPA Lysate)、HEK293T敲除细胞的Trizol裂解液(L15434 DCLK1 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。
- DCLK1基因的基本信息如下：

Species	Gene Symbol	Gene ID	GenBank Accession	Transcript
Human	DCLK1	9201	BC152456	NM_004734

About the gene	
Official Symbol	DCLK1
Previous Symbol	DCAMKL1
Official Full Name	doublecortin like kinase 1
Synonyms	KIAA0369; DCLK; DCDC3A
Location	13q13.3
Gene Type	protein_coding
Uniprot ID	O15075
Pathway/Library	Kinases Library
Gene Summary	This gene encodes a member of the protein kinase superfamily and the doublecortin family. The protein encoded by this gene contains two N-terminal doublecortin domains, which bind microtubules and regulate microtubule polymerization, a C-terminal serine/threonine protein kinase domain, which shows substantial homology to Ca ²⁺ /calmodulin-dependent protein kinase, and a serine/proline-rich domain in between the doublecortin and the protein kinase domains, which mediates multiple protein-protein interactions. The microtubule-polymerizing activity of the encoded protein is independent of its protein kinase activity. The encoded protein is involved in several different cellular processes, including neuronal migration, retrograde transport, neuronal apoptosis and neurogenesis. This gene is up-regulated by brain-derived neurotrophic factor and associated with memory and general cognitive abilities. Multiple transcript variants generated by two alternative promoter usage and alternative splicing have been reported, but the full-length nature and biological validity of some variants have not been defined. These variants encode different isoforms, which are differentially expressed and have different kinase activities.

<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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