

RPS6KA5 Knockout Lentivirus

产品编号	产品名称	包装
L16501	RPS6KA5 Knockout Lentivirus	10 ⁸ TU

产品简介:

- RPS6KA5 Knockout Lentivirus (RPS6KA5基因敲除慢病毒)是一种感染动物细胞后可以同时表达Cas9、目的基因sgRNA和puromycin抗性基因的慢病毒。本产品用于在动物细胞中基于CRISPR/Cas9技术敲除目的基因，并且本慢病毒中sgRNA的有效性已经通过T7EI法的验证。
- 本慢病毒基因序列的关键图谱信息请参考图1。本慢病毒可用于感染细胞或组织并进行目的基因的CRISPR/Cas9敲除。



图1. 可同时表达sgRNA、Cas9和puromycin抗性的本慢病毒其基因序列的关键图谱信息。

- 用于包装本慢病毒的质粒中的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T7EI法验证。
- 本慢病毒用于实验时，建议同时选购无任何靶向的对照慢病毒Control Knockout Lentivirus (L00015)或靶向GFP的对照慢病毒GFP Knockout Lentivirus (L00017)。
- 碧云天同时提供基于CRISPR/Cas9技术的RPS6KA5基因敲除的质粒(L16500 pLenti-RPS6KA5-sgRNA)、慢病毒(L16501 RPS6KA5 Knockout Lentivirus)、HEK293T细胞(L16502 RPS6KA5 Knockout HEK293T Cells)、HEK293T敲除细胞的RIPA裂解液(L16503 RPS6KA5 Knockout HEK293T RIPA Lysate)、HEK293T敲除细胞的Trizol裂解液(L16504 RPS6KA5 Knockout HEK293T Trizol Lysate)等产品，具体请在碧云天网站查询或在本产品网页点击相应产品。
- RPS6KA5基因的基本信息如下:

Species	Gene Symbol	Gene ID	GenBank Accession	Transcript
Human	RPS6KA5	9252	BC017187	NM_004755

About the gene	
Official Symbol	RPS6KA5
Previous Symbol	-
Official Full Name	ribosomal protein S6 kinase A5
Synonyms	MSK1; RLPK
Location	14q32.11
Gene Type	protein-coding gene
Uniprot ID	O75582
Pathway/Library	Kinases Library
Gene Summary	Serine/threonine-protein kinase that is required for the mitogen or stress-induced phosphorylation of the transcription factors CREB1 and ATF1 and for the regulation of the transcription factors RELA, STAT3 and ETV1/ER81, and that contributes to gene activation by histone phosphorylation and functions in the regulation of inflammatory genes (PubMed:11909979, PubMed:12569367, PubMed:12763138, PubMed:9687510, PubMed:18511904, PubMed:9873047). Phosphorylates CREB1 and ATF1 in response to mitogenic or stress stimuli such as UV-C irradiation, epidermal growth factor (EGF) and anisomycin (PubMed:11909979, PubMed:9873047). Plays an essential role in the control of RELA transcriptional activity in response to TNF and upon glucocorticoid, associates in the cytoplasm with the glucocorticoid receptor NR3C1 and contributes to RELA inhibition and repression of inflammatory gene expression (PubMed:12628924, PubMed:18511904). In skeletal myoblasts is required for phosphorylation of RELA at 'Ser-276' during oxidative stress (PubMed:12628924). In erythropoietin-stimulated cells, is necessary for the 'Ser-727' phosphorylation of STAT3 and regulation of its transcriptional potential (PubMed:12763138). Phosphorylates ETV1/ER81 at 'Ser-191' and 'Ser-216', and thereby regulates its ability to stimulate transcription, which may be important during development and breast tumor formation

	(PubMed:12569367). Directly represses transcription via phosphorylation of 'Ser-1' of histone H2A (PubMed:15010469). Phosphorylates 'Ser-10' of histone H3 in response to mitogenics, stress stimuli and EGF, which results in the transcriptional activation of several immediate early genes, including proto-oncogenes c-fos/FOS and c-jun/JUN (PubMed:12773393). May also phosphorylate 'Ser-28' of histone H3 (PubMed:12773393). Mediates the mitogen- and stress-induced phosphorylation of high mobility group protein 1 (HMGN1/HMG14) (PubMed:12773393). In lipopolysaccharide-stimulated primary macrophages, acts downstream of the Toll-like receptor TLR4 to limit the production of pro-inflammatory cytokines (By similarity). Functions probably by inducing transcription of the MAP kinase phosphatase DUSP1 and the anti-inflammatory cytokine interleukin 10 (IL10), via CREB1 and ATF1 transcription factors (By similarity). Plays a role in neuronal cell death by mediating the downstream effects of excitotoxic injury (By similarity). Phosphorylates TRIM7 at 'Ser-107' in response to growth factor signaling via the MEK/ERK pathway, thereby stimulating its ubiquitin ligase activity (PubMed:25851810). KS6A5_HUMAN,O75582
--	--

包装清单:

产品编号	产品名称	包装
L16501	RPS6KA5 Knockout Lentivirus	10 ⁸ TU
—	说明书	1份

保存条件:

-80°C保存, 至少一年有效。

注意事项:

- 碧云天拥有sgRNA序列的知识产权, 如果需要sgRNA序列, 请在订购后发送邮件向info@beyotime.com索取。sgRNA序列信息与本慢病毒, 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。使用者在发表研究论文或结果时, 应注明来源。
- 对于非目录产品的CRISPR基因敲除用的慢病毒的定制, 可联系碧云天技术服务service@beyotime.com。
- 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. 慢病毒的感染:

- a. 确定puromycin的筛选浓度: 待感染的细胞按一定密度铺在12孔或24孔中, 按照0、0.2、0.5、1、1.5、2、3、4、5μg/ml这样的浓度测试细胞对puromycin的敏感性, 推荐使用碧云天的Puromycin Dihydrochloride (嘌呤霉素) (ST551)。两天后细胞全部死亡的最低浓度即为该细胞的puromycin筛选浓度, 具体步骤参考碧云天该产品的使用说明: <https://www.beyotime.com/product/ST551-10mg.htm>。
- b. 慢病毒感染细胞: 按实验需要将细胞铺板(如12孔板), 细胞数以第2天密度约50%为宜。设置非感染细胞组、对照组和基因敲除组。37°C培养过夜后, 培养液中加入5~10μg/ml的Polybrene (C0351/ST551)。病毒感染前, 从-80°C冰箱取出病毒后冰浴融化, 参考相关文献或者根据预实验得到的MOI值加入适量病毒, 对于未浓缩的病毒, 可以直接按0.5ml/孔加入细胞, 对于浓缩或测定滴度的病毒, 一般100μl/孔或10⁷ TU已经足够, 轻轻摇匀, 37°C继续培养。两天后, 吸除含病毒的培养液, 换为新鲜的含一定浓度的puromycin的培养液进行筛选, 一般筛选2天后, 非感染细胞组细胞逐渐死去, 加入病毒组存活率比较高, 就可以收集部分细胞检测目的蛋白的表达或进行其它实验。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200μl接种到96孔板中(每孔平均0.5个细胞), 筛选单克隆细胞株。病毒感染的方法可参考Polybrene (C0351)的使用说明 <https://www.beyotime.com/product/C0351-1ml.htm>

2. 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
L00015	Control Knockout Lentivirus	10 ⁸ TU
L00017	GFP Knockout Lentivirus	10 ⁸ TU
C0222	青霉素-链霉素溶液(100X)	100ml
C0351-1ml	Polybrene (Hexadimethrine Bromide)	1ml
C0351-50mg	Polybrene (Hexadimethrine Bromide)	50mg

D0508S/M	基因组编辑突变检测试剂盒	25/100次
D7080S/M/L	T7 Endonuclease I (CRISPR等基因突变鉴定用)	250/1250/5000U
ST551-10mg	Puromycin Dihydrochloride (嘌呤霉素)	10mg/ml×1ml
ST551-50mg	Puromycin Dihydrochloride (嘌呤霉素)	10mg/ml×5ml
ST551-250mg	Puromycin Dihydrochloride (嘌呤霉素)	250mg
ST1380-500mg	Polybrene (≥94%, Reagent grade)	500mg
ST1380-2g	Polybrene (≥94%, Reagent grade)	2g
ST1380-10g	Polybrene (≥94%, Reagent grade)	10g

Version 2020.12.08