

## FFAR3 Knockout Lentivirus

产品编号	产品名称	包装
L26656	FFAR3 Knockout Lentivirus	10 <sup>8</sup> TU

### 产品简介:

- FFAR3 Knockout Lentivirus (FFAR3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FFAR3基因敲除的质粒(L26655 pLenti-FFAR3-sgRNA)、慢病毒(L26656 FFAR3 Knockout Lentivirus)、HEK293T细胞(L26657 FFAR3 Knockout HEK293T Cells)、HEK293T敲除细胞的RIPA裂解液(L26658 FFAR3 Knockout HEK293T RIPA Lysate)、HEK293T敲除细胞的Trizol裂解液(L26659 FFAR3 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。
- FFAR3基因的基本信息如下:

Species	Gene Symbol	Gene ID	GenBank Accession	Transcript
Human	FFAR3	2865	BC035657	NM_005304

About the gene	
Official Symbol	FFAR3
Previous Symbol	GPR41
Official Full Name	free fatty acid receptor 3
Synonyms	FFA3R
Location	19q13.12
Gene Type	protein-coding gene
Uniprot ID	O14843
Pathway/Library	others
Gene Summary	G protein-coupled receptor that is activated by a major product of dietary fiber digestion, the short chain fatty acids (SCFAs), and that plays a role in the regulation of whole-body energy homeostasis and in intestinal immunity. In omnivorous mammals, the short chain fatty acids acetate, propionate and butyrate are produced primarily by the gut microbiome that metabolizes dietary fibers. SCFAs serve as a source of energy but also act as signaling molecules. That G protein-coupled receptor is probably coupled to the pertussis toxin-sensitive, G(i/o)-alpha family of G proteins. Its activation results in the formation of inositol 1,4,5-trisphosphate, the mobilization of intracellular calcium, the phosphorylation of the MAPK3/ERK1 and MAPK1/ERK2 kinases and the inhibition of intracellular cAMP accumulation (PubMed:12711604). Activated by SCFAs and by beta-hydroxybutyrate, a ketone body produced by the liver upon starvation, it inhibits N-type calcium channels and modulates the activity of sympathetic neurons through a signaling cascade involving the beta and gamma subunits of its coupled G protein, phospholipase C and MAP kinases. Thereby, it may regulate energy expenditure through the control of the sympathetic nervous system that controls for instance heart rate. Upon activation by SCFAs accumulating in the intestine, it may also signal to the brain via neural circuits which in turn would regulate intestinal

	gluconeogenesis. May also control the production of hormones involved in whole-body energy homeostasis. May for instance, regulate blood pressure through renin secretion. May also regulate secretion of the PYY peptide by enteroendocrine cells and control gut motility, intestinal transit rate, and the harvesting of energy from SCFAs produced by gut microbiota. May also indirectly regulate the production of LEP/Leptin, a hormone acting on the CNS to inhibit food intake, in response to the presence of short-chain fatty acids in the intestine. Finally, may also play a role in glucose homeostasis. Besides its role in energy homeostasis, may play a role in intestinal immunity. May mediate the activation of the inflammatory and immune response by SCFAs in the gut, regulating the rapid production of chemokines and cytokines by intestinal epithelial cells. Among SCFAs, the fatty acids containing less than 6 carbons, the most potent activators are probably propionate, butyrate and pentanoate while acetate is a poor activator (PubMed:12496283, PubMed:12711604). FFAR3_HUMAN,O14843
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### 包装清单:

产品编号	产品名称	包装
L26656	FFAR3 Knockout Lentivirus	10 <sup>8</sup> 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:

- 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>。
- 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10<sup>7</sup> 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:

- 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。
- 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 <sup>8</sup> TU
L00017	GFP Knockout Lentivirus	10 <sup>8</sup> 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

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