

pLenti-RAB44-sgRNA

| 产品编号 | 产品名称 | 包装 |
|--------|--------------------|-----|
| L13520 | pLenti-RAB44-sgRNA | 5μg |

产品简介:

- pLenti-RAB44-sgRNA (RAB44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RAB44基因敲除的质粒(L13520 pLenti-RAB44-sgRNA)、慢病毒(L13521 RAB44 Knockout Lentivirus)、HEK293T细胞(L13522 RAB44 Knockout HEK293T Cells)、HEK293T敲除细胞的RIPA裂解液(L13523 RAB44 Knockout HEK293T RIPA Lysate)、HEK293T敲除细胞的Trizol裂解液(L13524 RAB44 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。
- RAB44基因的基本信息如下:

| Species | Gene Symbol | Gene ID | GenBank Accession | Transcript |
|---------|-------------|---------|-------------------|--------------|
| Human | RAB44 | 401258 | - | NM_001257357 |

| About the gene | |
|--------------------|--|
| Official Symbol | RAB44 |
| Previous Symbol | RASD3; RASL13 |
| Official Full Name | RAB44, member RAS oncogene family |
| Synonyms | dJ431A14.3 |
| Location | 6p21.2 |
| Gene Type | protein_coding |
| Uniprot ID | Q7Z6P3 |
| Pathway/Library | Lung Cancer Growth Related Genes Library |
| Gene Summary | Rab44 is an atypical Rab GTPase that contains some additional domains such as the EF-hand and coiled-coil domains as well as Rab-GTPase domain. Rab44 was an upregulated protein during osteoclast differentiation. Knockdown of Rab44 by small interfering RNA promotes RANKL-induced osteoclast differentiation of the murine monocytic cell line, RAW-D or of bone marrow-derived macrophages (BMMs). In contrast, overexpression of Rab44 prevents osteoclast differentiation. Rab44 was localized in the Golgi complex and lysosomes, and Rab44 overexpression caused an enlargement of early endosomes. A series of deletion mutant studies of Rab44 showed that the coiled-coil domain and lipidation sites of Rab44 is important for regulation of osteoclast differentiation. Mechanistically, Rab44 affects nuclear factor of activated T-cells c1 (NFATc1) signaling in RANKL-stimulated macrophages. Moreover, Rab44 depletion caused an elevation in intracellular Ca ²⁺ transients upon RANKL stimulation, and particularly regulated lysosomal Ca ²⁺ influx. It was suggested that Rab44 negatively regulates osteoclast differentiation by modulating intracellular Ca ²⁺ levels followed by NFATc1 activation. |

<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 |

Version 2020.12.09