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Ad-mCherry-GFP-LC3B

产品编号	产品名称	包装
C3011-1ml	Ad-mCherry-GFP-LC3B	1ml
C3011-10ml	Ad-mCherry-GFP-LC3B	10ml

产品简介:

- Ad-mCherry-GFP-LC3B, 即adenovirus expressing mCherry-GFP-LC3B fusion protein, 是一种可以表达mCherry-GFP-LC3B融合蛋白的腺病毒, 可以用于感染细胞或组织后进行细胞自噬(autophagy)的检测。
- LC3是酵母自噬关键蛋白ATG8在哺乳动物中的同源蛋白。LC3最初被分析鉴定为microtubule-associated protein 1 light chain 3 (MAP1LC3)。LC3蛋白家族包括LC3A、B、C和GABARAP等亚家族, 其中对于LC3B的研究最为广泛。到目前为止, LC3B被认为是细胞自噬信号通路最为关键的标志性蛋白。LC3B是一个具有125个氨基酸残基的蛋白, 在蛋白合成后, 被Atg4所酶切而失去了C端的22个氨基酸蛋白, 从而暴露出C端的甘氨酸, 人们把它命名为胞质形式的LC3B I。在细胞自噬的过程中, 其C端暴露的甘氨酸经过一个类似泛素化的过程, 以ATG7为E1样激活酶(E1-like activating enzyme), 以ATG3为E2样连接酶(E2-like conjugation enzyme), 以Atg12-Atg5-Atg16复合物为E3样连接酶(E3-like ligase), 在C端甘氨酸上共价连接上磷脂酰乙醇胺(phosphatidylethanolamine, PE)而成为LC3B-PE即LC3B II。与胞质定位的LC3B I不同, LC3B II定位于自噬体(autophagosome)的内膜和外膜上。在自噬体与溶酶体融合后, 自噬体外膜上的LC3B II被Atg4所酶切, 而自噬体内膜上的LC3B II则被溶酶体内的蛋白酶所降解。尽管LC3B II比LC3B I的分子量要大, 但由于其极强的疏水性, 在SDS-PAGE电泳时, LC3B II比LC3B I迁移得更快, 其表观分子量分别为14kD和16kD。自噬(Autophagy)是一种在进化上高度保守的通过溶酶体吞噬并降解部分自身组分的细胞内分解代谢途径。自噬与多种生理功能有关, 在饥饿等不利的环境条件下, 细胞通过自噬降解多余或异常的细胞内组分, 为细胞的生存提供能量及原材料, 促进生物体的生长发育、细胞分化及对环境变化产生应答。自噬异常与多种病理过程如肿瘤、神经退行性疾病、代谢疾病、病原体感染等都有密切关系。由于细胞自噬在生理和病理过程中都有重要作用, 自噬已经成为细胞生物学领域的一个新的研究热点。
- Ad-mCherry-GFP-LC3B是碧云天自行研发的重组腺病毒, 感染后能够在靶细胞中有效表达红色荧光蛋白mCherry、绿色荧光蛋白(GFP)和LC3B的融合蛋白, 呈现明亮的红色和绿色荧光, 可以用于细胞自噬的检测。
- 自噬小体在与溶酶体融合过程中, 溶酶体内的酸性环境会导致GFP荧光淬灭, 这为追踪GFP-LC3B的细胞定位增加了难度。mCherry是一种来自于蘑菇珊瑚(mushroom coral)的单体红色荧光蛋白, 当其与GFP进行LC3B的共同标记时, 在GFP被溶酶体酸性环境所淬灭的情况下, 可以发出红色荧光的mCherry因其卓越的稳定性而被保留。因此, 通过融合表达mCherry-GFP-LC3B蛋白, 可以非常有效地追踪自噬过程。在用Ad-mCherry-GFP-LC3B腺病毒感染细胞后, 在非自噬的情况下, 荧光显微镜下mCherry-GFP-LC3B以弥散的黄色荧光(mCherry和GFP的综合效果)形式存在于细胞质中; 而在自噬的情况下, 荧光显微镜下mCherry-GFP-LC3B则聚集在自噬体膜上, 以黄色斑点的形式表现出来(LC3B dot or punctae); 当自噬体与溶酶体融合后, 因GFP荧光的部分淬灭而以红色斑点的形式表现出来。
- 腺病毒感染细胞后为瞬时表达, 通常不会与基因组DNA重组, 不能用于筛选稳定细胞株。本产品感染体内外细胞后, 有效表达时间通常不少于7天, 不同细胞和组织的有效表达时间有所不同。感染10-14天后融合蛋白的表达很可能会大大减弱。
- 碧云天的Ad-mCherry-GFP-LC3B采用了成熟的E1缺陷型重组腺病毒载体系统, 感染普通的细胞后不能进行扩增和重组, 从而可以有效降低本产品在读体生物中的风险。
- 本产品可以在表达E1的HEK293A、HEK293等适当细胞中扩增。
- 本产品的滴度 $\geq 1 \times 10^8$ pfu/ml, 使用时可以按照 10^8 pfu/ml进行计算。如果按照20 MOI感染6孔板的细胞, 每孔50万细胞计算, 每毫升本产品共可以感染10个孔; 如果按照20 MOI感染24孔板的细胞, 每孔10万细胞计算, 每毫升本产品共可以感染50个孔。如果MOI值提高, 则相应可以感染的孔数会减少; 如果MOI值降低, 则相应可以感染的孔数会增加。

包装清单:

产品编号	产品名称	包装
C3011-1ml	Ad-mCherry-GFP-LC3B	1ml
C3011-10ml	Ad-mCherry-GFP-LC3B	1ml $\times$ 10
—	说明书	1份

保存条件:

-80℃保存, 一年有效。-20℃保存, 1-2个月内有效。4℃保存, 一周内有效。

注意事项:

- 反复冻融会降低病毒滴度，如有必要请在收到本产品后分装保存。病毒融解后，如果在一周内使用，可以放置于4℃。如果-80℃保存时间超过一年，可能会导致滴度下降，此时建议重新测定病毒滴度。
- 腺病毒感染需要细胞表面的CAR受体，请确认拟使用的细胞或组织可以被腺病毒感染。
- 腺病毒对不同细胞的感染效率请参考下表。

Species	Cell line	Origin	Transduction efficiency
Human	293	Embryonic Kidney	95%
	MCF-10A	Mammary epithelium	51%
	MDA-MB-231	Malignant breast cancer cell	72%
	HepG2	Hepatocarcinoma	70%
	T24	Transitional cell carcinoma	37%
	TCCSUP	Transitional cell carcinoma	53%
	PC-3	Prostate cancer metastases site	67%
	DU145	Prostate cancer metastases site	86%
	LNCaP	Prostate cancer metastases site	69%
	Jurkat	T cell leukemia	2%
	HRT	Rectal tumor	5%
Murine	MT1A2	Mammary tumor transformed with SV40 T antigen	92%
	NIH 3T3	Mouse fibroblast	12%
	WEHI	Fibrosarcoma	10%
	AML12	Liver	28%
	MS-K68	Spleen	48%
	EL4	T cell	2%
Bovine	FBRT HE1	Fetal retina transformed with Had5 E1	90%
	MDBK	Kidney	70%
	FBK-34	Kidney transformed with BAd3 E1	25%
	BT-5705	Turbinate	28%
	EBL	Embryonic lung	8%
Porcine	FPRT HE1-5	Fetal retina transformed with HAd5 E1	91%
	PK-15	Kidney	76%
	PK-M12	Kidney cell transformed with SV40 T antigen	71%
	PT-K75	Turbinate	22%
	EPL	Embryonic lung	53%
	SBC-1765	Derived from buffy coat cells	73%

- 对于难感染的细胞，建议使用较高的MOI值(可以达到100、200或500MOI)同时使用促病毒感染试剂Polybrene（终浓度2~4 μg/ml)以提高腺病毒的感染效率。
- 本产品使用前请仔细阅读附录1《腺病毒使用安全规范》。本产品生物安全等级为Biosafety Level 1 (BSL-1)，没有确凿证据显示会导致健康成人产生疾病(Not known to consistently cause diseases in healthy adults)，可以按照常规的微生物实验操作要求进行操作(Standard microbiological practices)。
- 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. 感染条件的确定：

不同种类的细胞感染所需的MOI值是不同的，如果是初次使用腺病毒感染需要通过实验确定最佳的感染条件。

- 细胞培养(以6孔板NIH3T3细胞为例，其它培养板或培养皿参考6孔板进行操作)：感染前一天在6孔板中以5×10⁵/孔接种NIH3T3细胞，每孔加入2ml完全培养液(具体的接种数量由细胞大小和细胞生长速度而定)，使第二天病毒感染时细胞密度达到约50%左右。
- 按照MOI 分别为2、5、10、20、40，计算所需病毒量，计算方法请参考附录2。
- 冰上解冻病毒，混匀后备用。
- 从细胞培养箱中取出6孔板，在显微镜下确定每孔的细胞均生长良好。吸弃旧培养液，每孔加入1.2ml新鲜培养液，并分别加入特定MOI值的病毒液，同时设置未加入病毒的细胞孔作为对照组。须特别注意在病毒感染时加入尽量少的新鲜培养液，以提升病毒的感染效率。对于培养液体积比较小的多孔板，例如96孔板和48孔板，也可以先把病毒用培养液稀释至所需的MOI值后再加入。
- 感染后约24h，除去含有病毒的培养液，每孔加入2ml新鲜的完全培养液，继续培养24h后观察细胞生长状况及荧光蛋白表达

情况(参考图1)，以不显著影响细胞生长、荧光较强且感染效率便于荧光观察的MOI值和感染后时间为最佳条件。

注1：检测细胞自噬时并非感染效率越高越好，通常约20-70%的感染效率已经足以进行自噬检测，感染效率过高反而容易产生细胞毒性而干扰检测。

注2：通常在腺病毒感染细胞后24小时即可观察到荧光蛋白表达的出现，在48小时左右有较强的表达效果。

注3：对于感染腺病毒后出现较强细胞毒性的情况，可以尝试在感染后6-12h更换成新鲜的完全培养液。

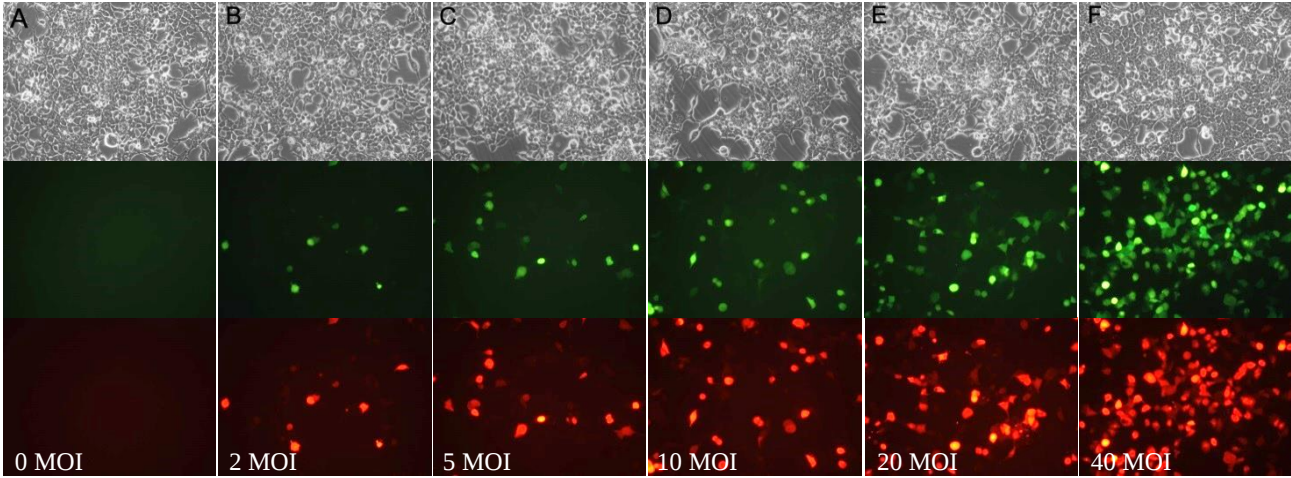


图1. Ad-mCherry-GFP-LC3B感染细胞的效果图。A、B、C、D、E和F依次为Ad-mCherry-GFP-LC3B分别在MOI值为0、2、5、10、20和40时感染NIH3T3细胞48h后的实拍效果图。上侧为明场照片，下侧为相同视野的荧光照片。

2. 感染细胞、诱导自噬和荧光观察

按照步骤1. 获得的条件进行感染实验，适当条件诱导自噬(如EBSS或者细胞自噬激活剂处理等)，随后可在荧光显微镜下观察LC3B的荧光变化情况。

附录：

1. 腺病毒使用安全规范

- 作为一种相对安全的病毒，尽管腺病毒基因组在感染后不会整合进入宿主细胞基因组中，也不会细胞体内进行复制，但是仍然具有可能的潜在生物学危险。我们建议使用者在病毒操作前应仔细阅读本规范，并在实验中严格按照本规范的要求进行操作。更为严格的美国CDC的生物安全等级及其操作与防护要求参考附表1，也可以访问如下网页：<https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf>。
- 腺病毒操作时应使用相应级别的生物安全柜，不同的腺病毒的生物安全等级会有所不同。如果使用普通超净工作台操作病毒，请不要打开排风机，以尽量避免可能污染病毒的尘埃正面吹向操作人员而被吸入。
- 实验操作时必需佩戴一次性帽子、口罩，穿戴实验手套及专门的实验服，避免身体直接接触病毒。手部及面部有开放性创口时，禁止进行病毒操作。
- 操作病毒时需小心谨慎，不要产生气雾或飞溅。如果操作时超净工作台或其它器皿上有病毒污染，请立即用70%乙醇或2%SDS溶液擦拭干净，或者采取其它的妥善措施。
- 如果需要离心，应使用密封性好的离心管，或用封口膜密封后离心，最好使用专门的离心机。
- 用显微镜观察细胞感染情况时应遵从以下步骤：拧紧培养瓶或盖紧培养板，用70%乙醇清理培养瓶或培养板外壁后到显微镜处观察拍照。离开显微镜实验台之前，用70%乙醇擦洗显微镜实验台。
- 所有被病毒污染过的枪头、离心管、培养板(皿、瓶)、培养液、手套等，在丢弃前请用84消毒液或2%SDS浸泡过夜。
- 脱掉手套后，用肥皂或洗手液清洗双手。

2. 病毒MOI的计算

MOI (Multiplicity of Infection)定义：病毒感染细胞时，病毒数量与细胞数量的比值。

Pfu (Plaque forming units)定义：具有生物活性的病毒颗粒数量。

可以根据如下公式计算所需pfu：

所需pfu=细胞数量×MOI

例如，需要向1×10⁵细胞中加入2 MOI的病毒，即所需pfu=(1×10⁵ cells)×(2 MOI)=2×10⁵ pfu。若病毒母液滴度为1×10⁸ pfu/ml，则细胞培养液中应加入(2×10⁵ pfu)/(1×10⁸ pfu/ml)=0.002ml病毒母液，即2μl病毒母液。

附表1. 生物安全等级及其操作与防护要求

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy	Standard microbiological	■ No primary barriers required.	Laboratory bench and sink required

	adults	practices	■ PPE: laboratory coats and gloves; eye, face protection, as needed	
2	■ Agents associated with human disease ■ Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: ■ Limited access ■ Biohazard warning signs ■ ‘Sharps’ precautions ■ Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: ■ BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials ■ PPE: Laboratory coats, gloves, face and eye protection, as needed	BSL-1 plus: ■ Autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	BSL-2 practice plus: ■ Controlled access ■ Decontamination of all waste ■ Decontamination of laboratory clothing before laundering	Primary barriers: ■ BSCs or other physical containment devices used for all open manipulations of agents ■ PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	BSL-2 plus: ■ Physical separation from access corridors ■ Self-closing, double-door access ■ Exhausted air not recirculated ■ Negative airflow into laboratory ■ Entry through airlock or anteroom ■ Hand washing sink near laboratory exit
4	■ Dangerous/exotic agents which post high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments ■ Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level ■ Related agents with unknown risk of transmission	BSL-3 practices plus: ■ Clothing change before entering ■ Shower on exit ■ All material decontaminated on exit from facility	Primary barriers: ■ All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit	BSL-3 plus: ■ Separate building or isolated zone ■ Dedicated supply and exhaust, vacuum, and decontamination systems ■ Other requirements outlined in the text

BSL, biosafety level; PPE, personal protective equipment.

相关产品:

产品编号	产品名称	包装
C3006-1ml	Ad-GFP-LC3B	1ml
C3006-10ml	Ad-GFP-LC3B	10ml
C3011-1ml	Ad-mCherry-GFP-LC3B	1ml
C3011-10ml	Ad-mCherry-GFP-LC3B	10ml
C3012-1ml	AdPlus-mCherry-GFP-LC3B	1ml
C3012-10ml	AdPlus-mCherry-GFP-LC3B	10ml

使用本产品的文献：

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Version 2024.03.12