

萤火虫萤光素酶报告基因检测试剂盒

产品编号	产品名称	包装
RG005	萤火虫萤光素酶报告基因检测试剂盒	100次
RG006	萤火虫萤光素酶报告基因检测试剂盒	1000次

产品简介:

- 碧云天生产的萤火虫萤光素酶报告基因检测试剂盒(Firefly Luciferase Reporter Gene Assay Kit), 是一种以萤光素(luciferin)为底物高信号稳定性检测萤火虫萤光素酶(firefly luciferase)活性的试剂盒。
- 本产品是萤火虫萤光素酶报告基因检测试剂盒II (RG007)的不同包装版本, 两者的检测效果完全一致。本产品, 即RG005/RG006为即用型液体, 其优点是无需配制即可直接使用, 但需要-80°C保存, 如果在-20°C保存时间较长后检测效果会逐渐下降。萤火虫萤光素酶报告基因检测试剂盒II, 即RG007, 为RG005/RG006的冻干粉版本, 优点是在-20°C保存非常稳定, 缺点是使用前需要使用提供的缓冲液充分溶解底物冻干粉后才能使用。
- **本产品的性能基本达到甚至在某些方面优于国外主要同类产品。**本产品的用途与碧云天的同类产品萤火虫萤光素酶报告基因检测试剂盒(增强型) (Enhanced Firefly Luciferase Reporter Gene Assay Kit)及Promega公司的Luciferase Assay System基本相同。本产品(RG005/RG006)的化学发光信号稳定性显著优于萤火虫萤光素酶报告基因检测试剂盒(增强型) (RG009)及国外同类产品(Competitor P) (图1B), 发光强度也可以达到萤火虫萤光素酶报告基因检测试剂盒(增强型) (RG009)及国外同类产品(Competitor P)的40% (图1A)。本产品与RG009及国外同类产品(Competitor P)的检测效果比较参见图1。

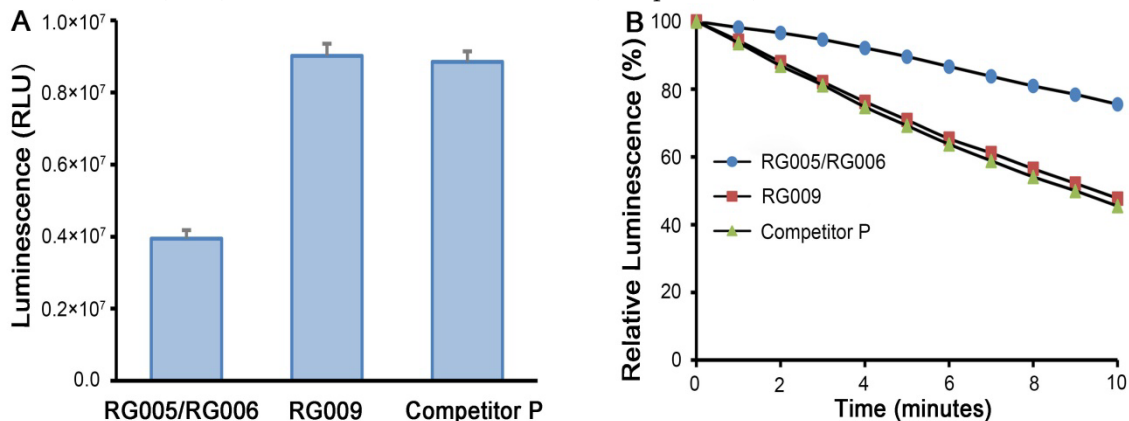


图1. 萤火虫萤光素酶报告基因检测试剂盒(RG005/RG006)的检测效果对比图。图中所示为本产品和碧云天的同类产品萤火虫萤光素酶报告基因检测试剂盒(增强型) (RG009)及国外同类产品(Competitor P)对转染阳性萤火虫萤光素酶报告基因质粒的HeLa细胞裂解样品的检测效果。图A为化学发光强度的检测效果对比图, 图B为化学发光稳定性的检测效果对比图。实际读数会因细胞种类、转染效率、报告基因质粒、检测仪器等的不同而存在差异, 图中数据仅供参考。

- **本产品发光信号稳定。**本产品的发光信号稳定性相对比较好, 1分钟内信号基本保持稳定, 信号变动不超过2%, 3分钟内的信号变动不超过5%, 5分钟内信号波动不超过10%, 信号半衰期约40分钟, 在发光稳定性方面显著优于RG009和国外同类产品Competitor P (图1B), 特别适用于待测样品数量较多的96孔板中进行萤火虫萤光素酶活性的多孔测定。
- **本产品发光强度高。**对于相同的细胞样品, 本产品的发光效果可以达到RG009和国外同类产品的约40%, 虽然低于RG009和国外同类产品(Competitor P), 但是发光强度相对已经非常高, 可以满足各种常规检测。如果样品中萤火虫萤光素酶的表达水平非常低时, 推荐使用发光强度和灵敏度更高的萤火虫萤光素酶报告基因检测试剂盒(增强型) (RG009/RG010)。
- **本产品操作简单, 读数稳定, 检测速度快, 从样品制备到完成检测仅需约20分钟。**本试剂盒中提供的萤火虫萤光素酶检测试剂为即用型试剂, 只需将100微升萤火虫萤光素酶检测试剂与20-100微升裂解制备的细胞样品混合后即可立即进行化学发光检测。并且发光信号比较稳定, 通常5分钟内信号下降不超过10%。
- **本产品稳定性好。**本试剂盒中的萤火虫萤光素酶检测试剂的稳定性非常好, 反复冻融5次对检测效果无明显影响, 反复冻融10次检测效果下降不超过10%。在4°C条件下, 保存3天检测效果下降不超过20%, 保存5天检测效果下降不超过30%, 保存7天仍可保留60%以上的检测效果。在室温保存1天可保留70%以上的检测效果, 室温保存3天可保留60%以上的检测效果, 37°C保存1天可保留50%以上的检测效果。萤火虫萤光素酶检测试剂即使仅保留约50%的检测效果, 仍可以满足各种常规检测的要求。
- 萤火虫萤光素酶是一种分子量约为61kD的蛋白, 在ATP、镁离子和氧气存在的条件下, 可以催化luciferin氧化成oxyluciferin。在luciferin氧化的过程中, 会发出生物萤光(bioluminescence)。生物萤光可以通过化学发光仪(luminometer)或液闪测定仪进

行测定[1]。本试剂盒的检测原理参考图2。

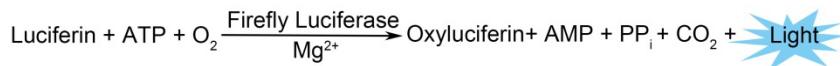


图 2. 萤火虫萤光素酶的检测原理图。

- 通过萤光素和萤光素酶这一生物发光体系，可以非常灵敏、高效地检测基因的表达。通常把感兴趣基因的转录调控元件或5'启动子区克隆在luciferase的上游，或把3'-UTR区克隆在luciferase的下游等，构建成报告基因(reporter gene)质粒。然后转染细胞，用适当药物等处理细胞后裂解细胞，测定萤光素酶活性。通过萤光素酶活性的高低来判断药物处理等对目的基因的转录调控作用[2]。
- 关于碧云天萤光素酶报告基因检测试剂盒相关产品的比较和选择，请参考碧云天的相关网页：<http://www.beyotime.com/support/luciferase-reporter-gene-assay.htm>
- 萤光素、萤光素酶、萤火虫萤光素酶和海肾萤光素酶也经常被称为荧光素、荧光素酶、萤火虫荧光素酶和海肾荧光素酶。
- 萤火虫萤光素酶催化luciferin发光的最强发光波长为560nm (centered around 560nm)。
- 本试剂盒RG005和RG006分别可以测定100个和1000个样品。

包装清单：

产品编号	产品名称	包装
RG005-1	报告基因细胞裂解液	60ml
RG005-2	萤火虫萤光素酶检测试剂	10ml
—	说明书	1份

产品编号	产品名称	包装
RG006-1	报告基因细胞裂解液	RG005-1×10
RG006-2	萤火虫萤光素酶检测试剂	RG005-2×10
—	说明书	1份

保存条件：

报告基因细胞裂解液4°C保存3个月有效，-20°C保存一年有效，-80°C可以长期保存；萤火虫萤光素酶检测试剂-80°C避光保存，至少一年有效；-20°C避光保存，推荐3-6个月内使用。

注意事项：

- 萤火虫萤光素酶检测试剂在-20°C保存其检测效果会逐渐下降，保存半年后其发光效果会降低约50%。因此，本产品如果保存于-20°C，推荐在3-6个月内使用。如果订购后可能放置较长时间后再使用，推荐订购在-20°C保存非常稳定的萤火虫萤光素酶报告基因检测试剂盒II (RG007)。
- 为取得最佳测定效果，在用单管的化学发光仪测定时，样品和测定试剂混合后到测定前的时间应尽量控制在相同时间内，例如30秒内；使用具有化学发光测定功能的多功能荧光酶标仪时，宜先把样品全部加好，然后统一加入萤火虫萤光素酶检测试剂。
- 由于萤光素酶的活性对温度比较敏感，所以反应前样品和检测试剂均需达到室温后再进行测定。可将萤火虫萤光素酶检测试剂在室温或不超过25°C的水浴中融解并混匀后使用。
- 尽管经测试萤火虫萤光素酶检测试剂反复冻融5次对其检测效果无明显影响，为保证萤光素酶检测试剂的稳定性、取得良好的使用效果，第一次解冻后可以采取适当分装后避光保存的方法，以避免反复冻融和长时间暴露于室温。
- 检测时需使用白色或黑色的96孔板。如果使用普通透明的96孔板，相邻孔之间会产生相互干扰。推荐使用碧云天的BeyoGold™全黑96孔细胞培养板(FCP966)或BeyoGold™全白96孔细胞培养板(FCP968)。
- 样品和测定试剂混合后，必须等待1-2秒，再进行测定。测定时间通常为10秒，根据情况也可以测定更长或更短时间，但是同一批样品宜使用相同的测定时间。
- 为避免由于质粒转细胞时效率的差异而带来的误差，可以同时转入海肾萤光素酶(Renilla luciferase)的报告基因质粒作为内参，采用碧云天的双萤光素酶报告基因检测试剂盒(RG027/RG028)进行检测；也可以同时转入β-半乳糖苷酶(β-galactosidase, β-gal)报告基因质粒作为内参，然后采用碧云天生产的β-半乳糖苷酶报告基因检测试剂盒(RG0036)进行检测。采用本试剂盒中的报告基因细胞裂解液裂解获得的样品可以直接用于β-半乳糖苷酶报告基因检测试剂盒(RG0036)的检测。
- 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. 裂解细胞：将报告基因细胞裂解液充分混匀后，按如下方式加入报告基因细胞裂解液，充分裂解细胞。

- 对于贴壁细胞：吸尽细胞培养液后，参考下表加入适量的报告基因细胞裂解液；对于悬浮细胞：离心去上清后，参考下表加入适量报告基因细胞裂解液。

器皿类型	96孔板	48孔板	24孔板	12孔板	6孔板
报告基因细胞裂解液 (微升/孔)	100	150	200	300	500

注：如果萤光素酶的表达水平比较低，可以尝试使用更少的裂解液，例如6孔板的每孔用量可以最小为100微升。

b. 充分裂解后, 10,000-15,000×g离心3-5分钟, 取上清用于测定。

注: 细胞裂解后可立即测定萤光素酶, 也可以先冻存, 待以后再测定。冻存样品需融解, 并达到室温后再进行测定。

2. 融解萤火虫萤光素酶检测试剂, 并达到室温。
3. 按仪器操作说明书开启化学发光仪或具有检测化学发光功能的多功能酶标仪, 可以将测定间隔设为2秒, 测定时间设为10秒, 或者根据仪器设备的要求并根据实验需要设置适当的间隔时间和测定时间。
4. 每个样品测定时, 取样品20-100微升(如果样品量足够, 请加入100微升; 如果样品量不足可以适当减少用量, 但同批样品的使用量宜保持一致), 取等体积的报告基因细胞裂解液作为空白对照。
5. 各孔加入100微升萤火虫萤光素酶检测试剂, 用枪打匀或用其它适当方式混匀后测定RLU (relative light unit)。本试剂盒的检测效果以及与同类竞争产品的检测效果比较可以参考图1。

常见问题:

1. Luminometer和荧光分光光度计有何不同?

荧光分光光度计检测的样品本身不能发光, 样品需要由特定波长的激发光激发, 然后才能产生荧光并被荧光分光光度计检测。Luminometer检测的样品本身可以发光, 不需要激发光进行激发。也就是说luminometer是检测化学发光(萤光)的仪器。有些型号的荧光分光光度计也具有luminometer的功能, 即也可以检测化学发光。您所使用的荧光分光光度计能否用于化学发光的测定请仔细阅读该仪器的说明书。

2. 可以进行ATP化学发光检测的仪器是否就可以用于本试剂盒的检测?

是。ATP化学发光的检测原理和本试剂盒的原理相同, 可以用相同的仪器测定。

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相关产品:

产品编号	产品名称	包装
RG005/RG006	萤火虫萤光素酶报告基因检测试剂盒	100/1000次
RG007S/M	萤火虫萤光素酶报告基因检测试剂盒II	100/1000次
RG009S/M	萤火虫萤光素酶报告基因检测试剂盒(增强型)	100/1000次
RG010S/M	萤火虫萤光素酶报告基因检测试剂盒II (增强型)	100/1000次
RG016/RG017	海肾萤光素酶报告基因检测试剂盒	100/1000次
RG027/RG028	双萤光素酶报告基因检测试剂盒	100/1000次
RG029S/M	双萤光素酶报告基因检测试剂盒II	100/1000次
RG051S/M	Bright-Lumi™萤火虫萤光素酶报告基因检测试剂盒	100/1000次
RG052S/M	Bright-Lumi™ II萤火虫萤光素酶报告基因检测试剂盒	100/1000次
RG055S/M	One-Lumi™萤火虫萤光素酶报告基因检测试剂盒	100/1000次
RG056S/M	One-Lumi™ II萤火虫萤光素酶报告基因检测试剂盒	100/1000次
RG058S/M	Steady-Lumi™萤火虫萤光素酶报告基因检测试剂盒	100/1000次
RG059S/M	Steady-Lumi™ II萤火虫萤光素酶报告基因检测试剂盒	100/1000次
RG062S/M	Renilla-Lumi™海肾萤光素酶报告基因检测试剂盒	100/1000次
RG066S/M	Renilla-Lumi™ Plus海肾萤光素酶报告基因检测试剂盒	100/1000次
RG088S/M	Dual-Lumi™双萤光素酶报告基因检测试剂盒	100/1000次
RG089S/M	Dual-Lumi™ II双萤光素酶报告基因检测试剂盒	100/1000次
RG126S/M	萤火虫萤光素酶报告基因细胞裂解液	10/100ml
RG127S/M	萤火虫萤光素酶报告基因细胞裂解液(增强型)	10/100ml
RG129S/M	海肾萤光素酶报告基因细胞裂解液	10/100ml
RG132S/M	双萤光素酶报告基因细胞裂解液	10/100ml

使用本产品的文献

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