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双萤光素酶报告基因检测试剂盒

产品编号	产品名称	包装
RG027	双萤光素酶报告基因检测试剂盒	100次
RG028	双萤光素酶报告基因检测试剂盒	1000次

产品简介:

- 碧云天生产的双萤光素酶报告基因检测试剂盒(Dual Luciferase Reporter Gene Assay Kit)，是先以萤光素(luciferin)为底物来检测萤火虫萤光素酶(Firefly luciferase)，后以肠腔素(coelenterazine)为底物来检测海肾萤光素酶(Renilla luciferase)，并且在后续加入海肾萤光素酶底物时，同时加入抑制萤火虫萤光素酶催化luciferin发光的物质，该物质可以淬灭约99.9%以上的萤火虫萤光素酶信号，使后续检测仅仅检测到海肾萤光素酶的活性，从而实现双萤光素酶报告基因检测。
- 本产品是双萤光素酶报告基因检测试剂盒II (RG029)的不同包装版本，两者的检测效果完全一致。本产品，即RG027/RG028中的萤火虫萤光素酶检测试剂为即用型液体，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。双萤光素酶报告基因检测试剂盒II (RG029)中的萤火虫萤光素酶检测试剂，为RG027/RG028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萤火虫萤光素酶检测缓冲液充分溶解底物冻干粉后才能使用。
- **本产品的性能总体优于国外主要同类产品**。本产品的用途与Promega公司的Dual-Luciferase® Reporter Assay System基本相同。本产品的检测灵敏度显著优于国外同类产品(Competitor P)，萤火虫萤光素酶的信号强度比国外同类产品(Competitor P)提高了约40%，海肾萤光素酶的信号强度比同类产品(Competitor P)提高了约25% (图1A、1B)；萤火虫萤光素酶的化学发光的信号稳定性显著优于国外同类产品(Competitor P) (图1C)，海肾萤光素酶的化学发光的信号稳定性与国外同类产品(Competitor P)基本一致或略优(图1D)；本产品的海肾萤光素酶检测工作液对萤火虫萤光素酶的淬灭效果较国外同类产品(Competitor P)更佳(图1A)。本产品与国外同类产品(Competitor P)的检测效果比较参见图1。

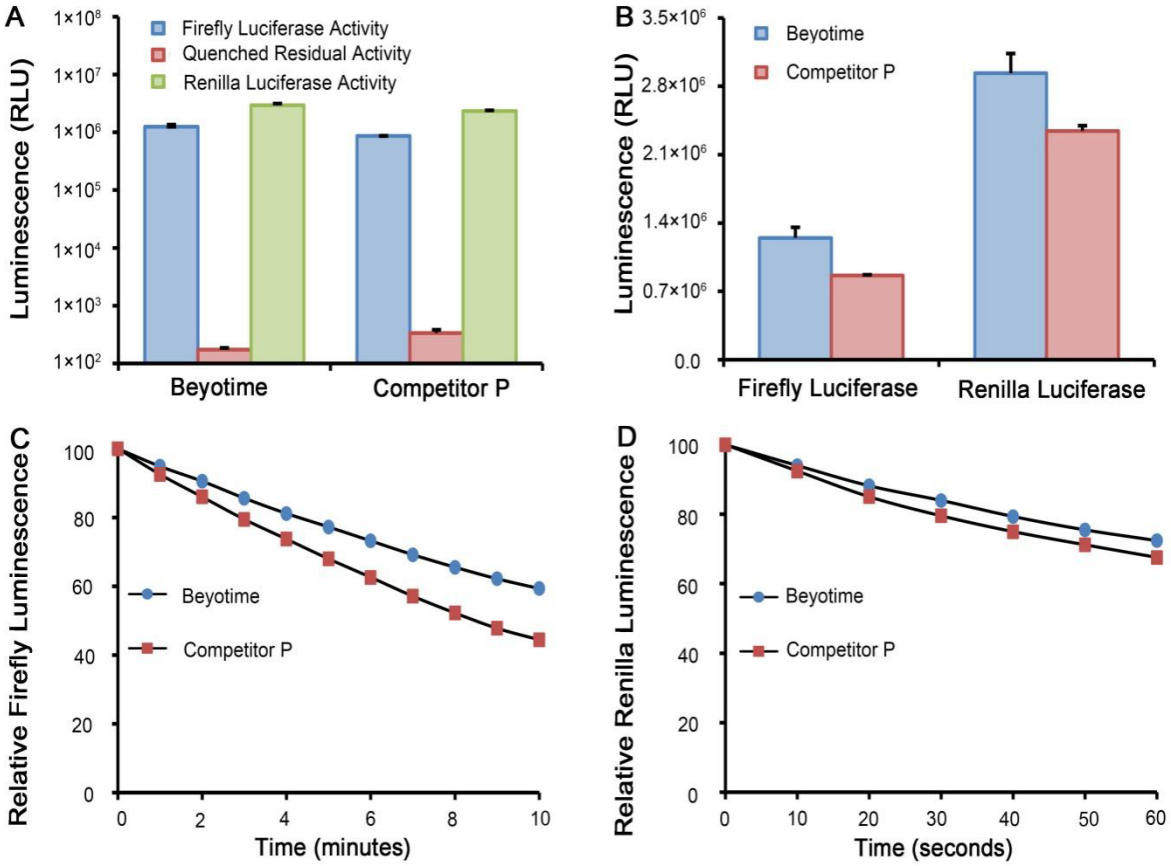

图1. 双萤光素酶报告基因检测试剂盒(RG027/RG028)的检测效果对比图。图中所示为本产品和国外同类产品(Competitor P)对共转染萤火虫萤光素酶报告基因质粒和海肾萤光素酶报告基因质粒的HeLa细胞裂解样品的检测效果。图A为整体检测效果对比图，图B为萤火虫萤光素酶和海肾萤光素酶的化学发光强度的检测效果对比图，图C为萤火虫萤光素酶的化学发光稳定性的检测效果对

比图，图D为海肾萤光素酶的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。

- **本产品发光强度高**。对于相同的样品，对萤火虫萤光素酶的检测，本产品的发光效果比国外同类产品(Competitor P)高约30-45%，对海肾萤光素酶的检测，本产品的发光效果比国外同类产品(Competitor P)高约20-30% (图1A、B)。
- **本产品操作简单，读数稳定，检测速度快，从样品制备到完成整个检测过程仅需约25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，海肾萤光素酶检测底物和海肾萤光素酶检测缓冲液按照1:100的比例混合即可配制成海肾萤光素酶检测工作液，再各取100微升先后与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。并且化学发光比较稳定，萤火虫萤光素酶的信号半衰期约15分钟，海肾萤光素酶的信号半衰期约200秒。
- **本产品的海肾萤光素酶检测工作液对萤火虫萤光素酶的淬灭效果好**。本试剂盒中的海肾萤光素酶检缓冲液和海肾萤光素酶检测底物配制的海肾萤光素酶检测工作液含有抑制萤火虫萤光素酶催化luciferin发光的物质，海肾萤光素酶检测试剂加入后通过简单的混匀，就可以抑制99.9%以上的萤火虫萤光素酶催化的发光信号，大大提高了后续海肾萤光素酶活性检测的精准性。
- **本产品稳定性好**。本试剂盒中的萤火虫萤光素酶检测试剂、海肾萤光素酶检测缓冲液和海肾萤光素酶检测底物(100X)的稳定性均较好。萤火虫萤光素酶检测试剂反复冻融5次对检测效果基本无影响，反复冻融10次检测效果下降不超过10%；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；室温保存1天可保留70%以上的检测效果，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。海肾萤光素酶检测缓冲液反复冻融10次、4°C和室温保存3天、37°C保存1天对检测效果的影响均不超过5%。海肾萤光素酶检测缓冲液反复冻融10次、4°C或室温保存3天对检测效果基本无影响，37°C保存1天对检测效果的影响不超过5%。海肾萤光素酶检测底物(100X)在4°C保存1周、室温保存1天检测效果下降不到10%，室温保存3天、37°C保存1天，仍可保留80%以上的活力。
- 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) [1]。海肾萤光素酶是一种分子量约为36kD的蛋白，在氧气存在的条件下，可以催化coelenterazine氧化成coelenteramide，在coelenterazine氧化的过程中也会发出生物萤光。生物萤光可以通过化学发光仪(luminometer)或液闪测定仪进行测定[2]。本试剂盒的检测原理参考图2.

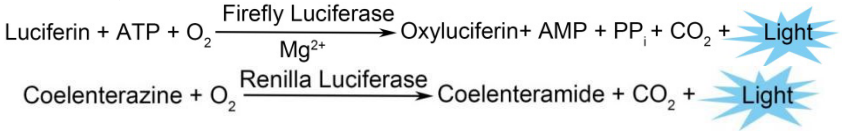

图 2. 双萤光素酶的检测原理图。

- 通过萤光素酶和其底物这一生物发光体系，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。Renilla luciferase相对更多地被用作转染效率的内参，以消除细胞数量和转染效率的差异[3]。
- 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作荧光素、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。海肾萤光素酶催化coelenterazine发光的最强发光波长为465nm。
- 本试剂盒RG027和RG028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
RG027-1	报告基因细胞裂解液	60ml
RG027-2	萤火虫萤光素酶检测试剂	10ml
RG027-3	海肾萤光素酶检测缓冲液	10ml
RG027-4	海肾萤光素酶检测底物(100X)	100μl
—	说明书	1份

产品编号	产品名称	包装
RG028-1	报告基因细胞裂解液	RG027-1×10
RG028-2	萤火虫萤光素酶检测试剂	RG027-2×10
RG028-3	海肾萤光素酶检测缓冲液	RG027-3×10
RG028-4	海肾萤光素酶检测底物(100X)	RG027-4×10
—	说明书	1份

保存条件：

报告基因细胞裂解液和海肾萤光素酶检测缓冲液在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。海肾萤光素酶检测底物(100X)在-20°C避光保存6

个月有效，-80°C避光保存一年有效。

注意事项：

- 本试剂盒中的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保存非常稳定的双萤光素酶报告基因检测试剂盒II (RG029)。
- 加入海肾萤光素酶检测工作液后对于上一步骤中的萤火虫萤光素酶的抑制可以达到约99.9%以上。但总会残留微量活性，因此，宜在转染时把海肾萤光素酶的表达量控制在其RLU读数高于萤火虫萤光素酶RLU读数的10%。海肾萤光素酶的读数高于萤火虫萤光素酶的读数是完全可以的，通常不会有明显的负面影响。
- 本试剂盒的海肾萤光素酶检测缓冲液在反复冻融过程中，可能会导致检测试剂中出现少量沉淀，此时宜平衡至室温，并尽量溶解。如仍有残留的不溶物，混匀后直接使用，经测试通常不会影响后续的检测效果。
- 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的时间内，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和海肾萤光素酶检测缓冲液在室温或不超过25°C的水浴中融解并混匀后使用。
- 尽管经测试本试剂盒中的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，为保证萤火虫萤光素酶检测试剂的稳定性、取得良好的检测效果，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。
- 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。
- 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)。
- RG027-4 海肾萤光素酶检测底物(100X)配制在无水乙醇中。由于无水乙醇容易挥发，有时会在初次使用时发现体积明显小于100μl的情况，此时用无水乙醇把体积补足至100μl，并混匀后即可使用。
- 海肾萤光素酶检测工作液宜配制后立即使用。如不能立即使用，-20°C可以保存一周。随着保存时间的延长检测效果会不断下降，因此不可配制成工作液后长期保存。
- 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。
   a. 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：细胞裂解后可以立即测定萤光素酶，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和海肾萤光素酶检测缓冲液，并达到室温。海肾萤光素酶检测底物(100X)置于冰浴或冰盒上备用。
3. 按照每个样品需100微升的量，取适量海肾萤光素酶检测缓冲液，按照1:100加入海肾萤光素酶检测底物(100X)配制成海肾萤光素酶检测工作液。例如，1毫升海肾萤光素酶检测缓冲液中加入10微升海肾萤光素酶检测底物(100X)充分混匀后即可配制成约1毫升海肾萤光素酶检测工作液。
4. 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。
5. 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。
6. 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)。
7. 在完成上述测定萤火虫萤光素酶步骤后，加入100微升海肾萤光素酶检测工作液，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可以参考图1。
8. 在以海肾萤光素酶为内参的情况下，用萤火虫萤光素酶测定得到的RLU值除以海肾萤光素酶测定得到的RLU值。根据得到的比值来比较不同样品间目的报告基因的激活程度。如果以萤火虫萤光素酶为内参，也可以进行类似计算。

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。

参考文献：

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2. Matthews J C, Hori K, Cormier M J. Biochemistry. 1977. 16(1):85-91.
3. E Schenborn, D Groskreutz. Mol Biotechnol. 1999. 13:29-44.

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

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