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EMSA探针生物素标记试剂盒

产品编号	产品名称	包装
GS008	EMSA探针生物素标记试剂盒	20次

产品简介：

- EMSA探针生物素标记试剂盒(EMSA Probe Biotin Labeling Kit)是一种通过Terminal Deoxynucleotidyl Transferase (TdT)把生物素标记的dUTP添加到单链DNA 3'末端，然后通过退火产生生物素标记的EMSA探针的试剂盒。通常DNA的3'末端被标记后不会干扰基于序列特异性蛋白结合的EMSA检测。
- Terminal Deoxynucleotidyl Transferase (TdT)可以在不依赖于模板的情况下催化在DNA的3'-OH端加上dNTP的反应。关于TdT催化双链DNA末端加dNTP的反应效率，3'端突出的双链DNA要比平端或3'端缩进的双链DNA高很多。TdT催化单链DNA末端加dNTP的反应效率要比双链DNA高很多。在适当的条件下，TdT也可以催化RNA 3'末端加NTP的反应。
- 本试剂盒适合标记纯化的单链DNA，如果用于标记EMSA探针，可以在单链标记后再进行退火。
- 本试剂盒中提供了已经用生物素标记好的Biotin-Control Oligo，可以用作检测DNA标记效率的对照。
- 如果每个标记反应的探针量为5pmol，本试剂盒可以用于20个标记反应。

包装清单：

产品编号	产品名称	包装
GS008-1	TdT Buffer (5X)	250μl
GS008-2	TdT (10U/μl)	20μl
GS008-3	Biotin-11-dUTP (5μM)	100μl
GS008-4	Biotin-Control Oligo (0.4μM)	100μl
GS008-5	Ultrapure water	1ml
GS008-6	探针标记终止液	200μl
GS008-7	TE	15ml
GS008-8	退火缓冲液(10X)	150μl
—	说明书	1份

保存条件：

-20°C保存。

注意事项：

- 需自备用于检测探针标记效率的带正电荷尼龙膜，以及用于生物素检测的相关试剂。带正电荷尼龙膜(FFN10/FFN11/FFN13/FFN15)可以向碧云天订购。相应的用于生物素检测的化学发光法EMSA试剂盒(GS009)也可以向碧云天订购。
- 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. 准备工作：

a. 取出TdT Buffer (5X)、Biotin-11-dUTP和Ultrapure water溶解，并置于冰浴上备用。

b. 取出待标记的单链EMSA探针，用水稀释至1μM，并置于冰浴上备用。如果待标记的EMSA探针为双链，95°C加热2分钟，然后立即放置到冰水浴中，使双链的EMSA探针转变为单链的探针，然后同样用水稀释至总的单链DNA浓度为1μM，即每条单链的浓度为0.5μM，相当于最初双链的EMSA探针浓度为0.5μM。

2. DNA探针的标记：

Ultrapure water	29μl
TdT Buffer (5X)	10μl
待标记探针(1μM)	5μl
Biotin-11-dUTP (5μM)	5μl
TdT (10U/μl)	1μl

总体积	50μl

a. 参考上表设置反应体系。注：对于双链的EMSA探针的标记反应，建议一次做两管，即总体积共100μl，以最终获得足够的生物素标记EMSA探针用于后续EMSA检测。
b. 用枪轻轻吹打混匀，切勿vortex。37°C孵育30分钟。
c. 加入2.5μl 探针标记终止液，轻轻混匀终止反应。

3. TdT的去除：

a. 探针标记反应终止后，加入52.5μl氯仿-异戊醇(24:1)，vortex使有机相和水相充分混合以抽提TdT(说明：静止后有机相和水相会很快分层)。
b. 12000-14000g离心1-2分钟。吸取上清备用。上清即为被生物素标记的单链DNA探针。

4. 探针的纯化(选做)：

通常为实验简便起见，可以不必纯化标记好的探针。有些时候，纯化后的探针会改善后续实验的结果。如需纯化，可以按照如下步骤操作：

a. 对于100μl标记好的探针，加入1/4体积即25μl的5M醋酸铵，再加入2体积即200μl的无水乙醇，混匀。
b. -70°C至-80°C沉淀1小时，或-20°C沉淀过夜。
c. 4°C，12,000g-16,000g离心30分钟。小心去除上清，切不可触及沉淀。
d. 4°C，12,000g-16,000g离心1分钟。小心吸去残余液体。微晾干沉淀，但不宜过分干燥。
e. 加入50μl TE，完全溶解沉淀。标记好的探针可以-20°C保存。

5. 生物素标记探针标记效率的检测：

a. 取5μl Biotin-Control Oligo (0.4μM), 加入196μl TE, 混匀, 稀释成10nM Biotin-Control Oligo(作为标准品)。取出适量10nM Biotin-Control Oligo，依次稀释成5nM、2.5nM、1nM、0.5nM和0.25nM。
b. 取3μl步骤3B所获得的生物素标记的DNA探针(100nM), 加入27μl TE, 混匀, 稀释成10nM 生物素标记的探针(作为待测样品)。取出适量的10nM 生物素标记的探针，依次稀释成5nM、2.5nM、1nM、0.5nM和0.25nM。
c. 参考下面的表格，取一适当大小的带正电荷尼龙膜，在膜上做好相应标记。对于经过梯度稀释的标准品和待测样品，分别取2μl滴加到膜上。在膜上滴加标准品或待测样品时，请注意使液滴充分被膜吸收，在膜上形成一个湿的圆形小斑点。说明：如果条件许可，可以使用专门用于点杂交或狭缝杂交的设备进行探针标记效率的检测，探针的用量参考下表，浓度可以再稀释50倍，而所用体积可以相应放大50倍至100μl。

探针浓度	10nM	5nM	2.5nM	1nM	0.5nM	0.25nM
Biotin-Control Oligo	2μl	2μl	2μl	2μl	2μl	2μl
生物素标记的探针	2μl	2μl	2μl	2μl	2μl	2μl
探针量	20fmol	10fmol	5fmol	2fmol	1fmol	0.5fmol

d. 滴加完所有的标准品和样品后，将膜室温晾干。
e. 用紫外交联仪(UV-light cross-linker)选择254nm紫外波长，120mJ/cm2，交联30-45秒。如果没有紫外交联仪可以使用普通的手提式紫外灯(例如碧云天的手提紫外检测仪(EUV002))，距离膜5-10厘米左右照射1-5分钟。也可以使用超净工作台内的紫外灯，距离膜5-10厘米左右照射1-10分钟。最佳的交联时间可以使用标准品自行摸索。
f. 随后可以立即采用各种生物素检测试剂盒，检测出样品的生物素标记效率；也可以室温存放数天直至进行后续检测。
g. 如果最后采用的是ECL类试剂或其它类似试剂进行检测，则可以对比样品和标准品的灰度，从而计算出探针的标记效率。例如2fmol量的待测样品探针的灰度和1fmol标准品的灰度相同，则说明探针的标记效率大致为50%，待测样品中总探针的浓度约为1μM，而实际被生物素标记的探针约为0.5μM。探针的标记效率也可以通过建立标准曲线进行比较精确的计算。用于后续检测时通常要求标记效率不低于30%。有文献报道标记效率和3'末端的碱基无关，但和整个待标记探针的序列有关。由于在TdT的催化下可以在待标记探针的3'端加上多个Biotin标记的dUTP，因此有时会出现标记效率大于100%的情况。

6. 生物素标记EMSA探针的制备：

a. 对于步骤3B标记好的单链DNA探针，把正义链和反义链等体积混合(不可根据标记效率调整摩尔比例)。对于最初使用变性的双链EMSA探针进行探针标记的情况，直接进入下一步。
b. 加入退火缓冲液(10X)，使退火缓冲液的最终浓度为1X，混匀。例如待退火探针的体积为100微升，则加入11微升退火缓冲液(10X)。
c. 如下设置PCR仪进行退火反应：

步骤	温度	时间	说明
1	95°C	2分钟	让oligo充分变性
2	每8秒下降0.1°C，降至25°C(注1)	约90分钟	退火
3	4°C	长时间保持	暂时存放

注1：如果所用的PCR仪不具备下降0.1°C的功能，也可以设置为每90秒下降1°C。

d. 退火反应结束后，-20°C保存标记好的EMSA探针。此时的EMSA探针已经可以直接用于后续的EMSA检测，也可以对探针进行适当纯化后再进行EMSA检测。

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