总 RNA 提取试剂

产品简介：

总 RNA 提取试剂(Total RNA Extraction Reagent)，亦称一步法 RNA 提取试剂。本产品含饱和酚和异硫盐等物质，能迅速裂解细胞或组织并且灭活核酸酶，保持 RNA 的完整性。加入氯仿并离心后，溶液形成上清层为水相(无色)、中间层、下层为有机相(红色)；上清层用异丙醇沉淀回收总 RNA，中间层用乙醇沉淀回收 DNA，下层用异丙醇沉淀回收蛋白。

本产品适用于从各种组织或细胞中快速分离总 RNA，既可用于小量样品(50～100 mg 组织、5×10^6 细胞)，也可用于大量样品(>1g 组织/>10^7 细胞)，提取的总 RNA 质量高，可用于 Northern blot、Dot blot、polyA 筛选、体外翻译、RNase 保护分析和分子克隆，该试剂具有以下特点：①适用范围广；②操作简单，整个过程 1 小时内完成；③纯度高；④污染少。该试剂仅用于科研领域，不适用于临床诊断或其他用途。

产品组成：

编号 / 名称	NR0002	Storage
总 RNA 提取试剂	100ml	4℃ 避光
使用说明书	1 份	

自备材料：

1、试剂：无水乙醇、异丙醇、DEPC 处理水、氯仿或氯仿替代物等。

2、耗材：RNase 广泛存在于人的皮肤上和体液及环境中，RNase 是导致 RNA 降解的最主要物质，非常稳定。操作时应佩戴一次性口罩、手套、帽子。塑料制品、玻璃和金属物品、实验仪器等应清除 RNase，移液器吸头、EP 管等制品的 RNase free 处理尤为重要。

3、仪器：低温高速离心机、低温冰箱。

操作步骤(仅供参考)**：**

1、样品准备

⑴贴壁细胞：

①直接裂解：直接在培养瓶/皿中加入总 RNA 提取试剂裂解细胞，每 10cm^2 面积加 1ml 总 RNA 提取试剂，用移液器吹打混匀。

②胰蛋白酶消化：用无菌 PBS 洗涤细胞后，加入含有 0.05～0.25%胰蛋白酶的 PBS 处理细胞，当细胞脱离容器壁后，加入含有血清的培养基终止反应，将细胞溶液转移至无 RNase 的离心管中，5000～6000g 离心 5 min，收集细胞沉淀，去除上清。收集细胞时一定要将细胞培养液去除干净，否则裂解不完全，降低 RNA 收获率。

⑵悬浮细胞：无需清洗细胞，直接 5000～6000 g 离心 5 min，收集细胞，每 5×10^6～10^7 动物、植物和酵母细胞或每 10^7 细菌细胞加入 1ml 总 RNA 提取试剂。

⑶组织：取新鲜动物或者植物组织或者-70℃冻存组织，50～100mg 组织在液氮中充分研磨或者加入 1ml 总 RNA 提取试剂研磨或者用匀浆器匀浆处理。样品体积一般不超过总 RNA 提取试剂体积的 10%，研磨要迅速，以 1min 为佳。

⑷血液：取 0.5～1 ml 新鲜或冻存的血液，12000g 离心 5 min，去除血浆，加入 1ml 总 RNA 提取试剂，充分振荡混匀。

2、核酸分离：充分振荡混匀(可以置于低温/超低温冰箱冻存 5～10 min 后，充分振荡，反复 1～3 次)，将裂解样品或匀浆液室温放置 5～10 min，使核蛋白与核酸完全分离。

3、样品分层：加入 0.2 ml 氯仿/1ml 总 RNA 提取试剂，剧烈振荡 15 s，室温放置 2～3 min，置于 4℃离心机，12000 g 离心 10～15 min；上层为水相，中间层和下层为有机相，RNA 在上层水相。
4、沉淀 RNA：吸取上层水相(约 500μl)转移至无 RNase 的离心管中(不要吸取任何中间层物质,否则会有染色体 DNA 污染)，加入等体积异丙醇混匀(或者加入 1.2 倍体积专用 RNA 沉淀液，超低温冰箱放置 2～3hr，可以大大提高 RNA 回收率)，室温放置 15～20 min，12000 g 4℃离心 10 min，离心后管侧或管底形成胶状沉淀，弃上清。
5、洗涤 RNA：加入 1ml DEPC 水配制的 75%乙醇洗涤沉淀，室温放置 5～10 min，7500g 4℃离心 5 min，弃上清，室温干燥 5～10 min，不宜过分干燥，否则 RNA 难以溶解。
6、溶解 RNA：加入 30～50ul RNase-free ddH_2O 充分溶解 RNA，-70℃长期保存或直接用于后续试验。对于肝、胰腺、肾等组织中 RNase 含量高的样品，沉淀时用 100%去离子甲酰胺溶解。

分析与定量：

1、测定样品在 260 nm 和 280 nm 的吸收值确定 RNA 的质量，$OD_{260/280}$ 在 1.8～2.0 视为抽提 RNA 纯度较好。
2、进行甲醛变性琼脂糖电泳，确定 RNA 的完整性和污染情况。
3、核酸分析仪测定 RNA 的质量和纯度。

注意事项：

1、样品保存：加入总 RNA 提取试剂混匀后，样品可在-70℃放置 1～2 月；RNA 样品可以在 70%酒精中-70℃保存 2～4 周：如果需要长期保存，应置于超低温冰箱中保存。
2、本品含强腐蚀性物质，污染皮肤或眼睛后，立即用清水或生理盐水冲洗，必要时寻求医生的帮助。
3、为了您的安全和健康，请穿实验服并戴一次性手套操作。

有效期：12 个月有效。常温运输，4℃避光保存。

常见问题分析：

常见问题	可能原因
A_{260}/ A_{280} < 1.6	抽提得到的 RNA 沉淀未完全溶解。
	水相中混有有机相，从而存在蛋白质和 DNA 污染。
	RNA 样品用水而不是 TE 溶解。低离子浓度和低 pH 条件下，A280 值会偏高。
	样品匀浆时加的总 RNA 提取试剂试剂太少，RNA 与蛋白质、DNA 未能完全分离。
	匀浆后样品未在室温放置或者放置时间太短，RNA 与核蛋白未完全解离。
DNA 污染	样品中含组织溶剂(如乙醇等)或碱性溶液，致水相减少或 pH 升高。
	样品匀浆时加入的试剂体积太少。如果存在 DNA 污染，可用 DNA 清除剂去除。
	水相中混有有机相，从而存在蛋白质和 DNA 污染。
RNA 产量低	样品裂解或匀浆处理不彻底，RNA 没有被完全释放出来。
	得到的 RNA 沉淀未完全溶解。
	抽提的 RNA 中含有 RNase。
RNA 降解	组织或细胞不新鲜，样品没有及时被液氮冻存，导致组织或细胞中的 RNA 降解。
	溶液或离心管未经 RNase free 处理，RNase 的污染导致 RNA 被降解。
	细胞在胰蛋白酶消化时间过长，导致未加总 RNA 提取试剂前 RNA 已经部分降解。
	电泳时使用的甲酰胺 pH 小于 3.5，导致 RNA 发生酸解。
蛋白和多糖污染	水相中混有有机相，从而带有蛋白质和 DNA。
	样品中蛋白、多糖含量高或样品量太大，细胞未裂解完全。

相关产品：

产品编号	产品名称
NA0006	DNA 凝胶上样缓冲液(6×DNA Loading Buffer)
NA0030	50×TAE 缓冲液
NA0034	5×TBE 缓冲液
NA0093	GoldView(10000×)
NA0095	GelRed(10000×)
NA0096	GelGreen(10000×)
NA0200	DNA Marker(100bp-1500bp)
ND0081	EDTA 溶液(0.5mol/L,pH8.0)
NE0011	CTAB 抽提液
NE0049	RNA 沉淀剂(75%,RNase free)
NE0092	水饱和酚(Phenol Water)
NE0095	Tris 饱和酚(pH > 7.8)
NE0120	溶菌酶(Lysozyme,10mg/ml)
NE0210	λ噬菌体基因组 DNA 提取试剂盒(PEG 沉淀法)
NE0260	组织细胞 RNA 提取试剂盒(一步提取法)
NE0301	核酸助沉剂(Glycogen,5mg/ml)
NE0310	Glycogen(核酸沉淀用)
NE0500	核酸提取辅助试剂(24:1 替代试剂)
NE0502	核酸提取辅助试剂(25:24:1 替代试剂,pH > 7.8)
NE0503	核酸提取辅助试剂(25:24:1 替代试剂,pH < 5.0)
NE0506	核酸提取辅助试剂(25:24 替代试剂,pH > 7.8)
NE0508	核酸提取辅助试剂(25:24 替代试剂,pH < 5.0)
NE0510	核酸提取辅助试剂(1:1 替代试剂,pH > 7.8)
NE0512	核酸提取辅助试剂(1:1 替代试剂,pH < 5.0)
NH0012	磷酸缓冲盐溶液(1×PBS,无钙镁,RNase free)
NH0043	SSC 缓冲液(20×,pH7.0)
NH0053	变性鲑鱼精 DNA(10mg/ml)
NI0001	RNase 抑制剂(40U/μl,鼠源)
NP0053	三磷酸腺苷溶液(ATP,10mmol/L)
NP0059	甜菜碱溶液(5mol/L,PCR 级)
NP0063	无菌去离子水(For PCR)
NP0211	2×HotStart qPCR Probe master mix
NR0001	DEPC 处理水(0.1%)
NR0002	总 RNA 提取试剂
NR0004	RNA 提取辅助试剂(氯仿替代物)
NR0005	DTT 溶液(1mol/L,RNase free)
NR0007	无酶无菌水(非 DEPC 处理)
NR0073	Tris-HCl 缓冲液(1mol/L,pH8.0,RNase free)
NR0095	去离子甲酰胺
NR0220	氯化镁溶液(1mol/L,RNase free)
NV0010	细菌冻存液
NZ0101	核酸清除剂
NZ0111	RNase and DNase Away
NZ0112	RNase, DNase and DNA Away
NZ0113	RNase, DNase, RNA and DNA Away

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