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RNAeasy™动物RNA抽提试剂盒(离心柱式)

产品编号	产品名称	包装
R0026	RNAeasy™动物RNA抽提试剂盒(离心柱式)	50次

产品简介:

- 碧云天的离心柱式RNAeasy™动物RNA抽提试剂盒(RNAeasy™ Animal RNA Isolation Kit with Spin Column)是一种基于离心柱法从动物组织或培养的动物细胞中安全、快速、便捷、稳定、高效、高质量地抽提长度大于200个核苷酸(nucleotide, nt)的RNA的试剂盒。抽提获得的大于200个核苷酸的RNA(也常被称为总RNA)可以用于各种常规用途。
- 本试剂盒抽提得到的RNA可用于反转录、RT-PCR、qPCR、Northern、点杂交(Dot Blot)、纯化mRNA、体外翻译、RNase protection assay、cDNA克隆等下游实验；也可用于基因表达芯片分析(microarray)、高通量测序(high throughput sequencing)等对RNA质量要求较高的情况。
- 碧云天的三款离心柱式及Trizol法RNA抽提试剂盒的主要特点和差异如下:

产品编号	R0024/ R0026/ R0027	R0028	R0032	R0011/R0016
产品名称	RNAeasy™动物RNA抽提试剂盒	RNAeasy™动物小RNA抽提试剂盒	RNAeasy™ Plus动物RNA抽提试剂盒	Beyozol Trizol
推荐指数	★★★★★	★★★★★	★★★	★★
抽提方式	离心柱式	离心柱式	离心柱式	原始方式
抽提产物	RNA(>200nt)	小RNA(<200nt)	总RNA (含<200nt的小RNA)	总RNA (含<200nt的小RNA)
操作时间	最短(15-20分钟)	稍较长(20-25分钟)	较长(25-30分钟)	最长(45-60分钟)
操作步骤	4步骤(**少**)	6步骤(**多**)	6步骤(**多**)	很多步骤
使用便捷性	★★★★★	★★★★	★★★★	★★
推荐细胞量/得率	50-100万/5-20μg	50万/1-2μg	50-100万/5-20μg	50-100万/5-20μg
推荐组织量/得率	5-20mg/10-40μg (**多**)	5mg/1-1.5μg (**少**)	3-5mg/3-10μg (**少**)	50mg/100-500μg (**很多**)
RNA纯度	高	高	高	高
有毒有害试剂	无	无	无	有
人体与环境安全	高	高	高	低
国外同类产品	RNeasy Mini Kit (Qiagen) PureLink® RNA Mini Kit (Thermo)	PureLink® miRNA Isolation Kit (Thermo)	miRNeasy Mini Kit (Qiagen)	TRIzol (Thermo)
用途	非小RNA相关各种用途	小RNA相关各种用途	不同长度RNA各种用途	不同长度RNA各种用途

- 动物组织或细胞中的RNA按照长度可以分为长链RNA(long RNA)和小RNA(small RNA)，长链RNA通常大于200nt，而小RNA通常小于200nt。长链RNA按照是否编码蛋白或多肽可以分为长链非编码RNA(long noncoding RNA, lncRNA)和mRNA。小RNA主要包括非编码的5.8S rRNA(ribosomal RNA)、5S rRNA、tRNA(transfer RNA)、microRNA(miRNA)、siRNA(small interfering RNA)、piRNA(Piwi-associated small RNA)、tsRNA(tRNA-derived small RNA)、srRNA(small rDNA-derived RNA)等。
- 本试剂盒抽提RNA的基本流程如图1所示。样品在裂解液中迅速裂解，释放出总RNA，然后让RNA特异性地结合到纯化柱上，而基因组DNA和蛋白质等其它组分通过高速离心被有效去除，再通过3次洗涤充分去除非特异性结合的蛋白、盐等杂质，最后用洗脱液将高纯度的RNA洗脱下来。

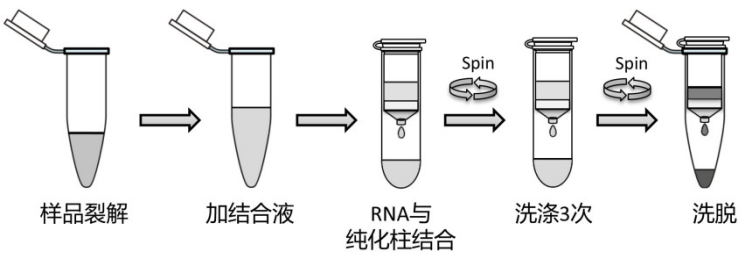
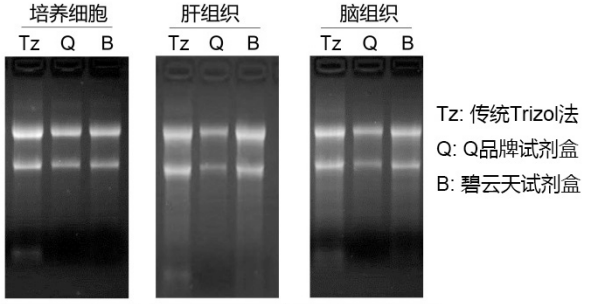


图1. 离心柱式RNA抽提流程示意图

- **本试剂盒使用安全**。本试剂盒通过特殊的柱纯化介质进行RNA分离纯化，能有效避免传统的Trizol法抽提时使用的酚、氯仿等有毒有害有机试剂。
- **本试剂盒使用快速、便捷**。本试剂盒采用柱纯化，无需繁琐的RNA沉淀步骤，抽提操作过程仅15-20分钟。相比于传统的Trizol抽提法，本试剂盒的操作流程显著简化，缩短了抽提时间，降低了RNA被降解的风险。和国外同类柱纯化产品相比，所需操作步骤和操作时间基本一致。
- **本试剂盒抽提RNA的得率高**，优于国外同类产品。本试剂盒的抽提效果经反复测试，100万个HeLa细胞能抽提得到约15-20μgRNA，20mg小鼠肝组织能抽提得到约25-35μgRNA，20mg小鼠脑组织中抽提得到约10-20μgRNA(新鲜样品的得率高于冻存样品)。本试剂盒与传统的Trizol方法及国际知名Q品牌同类产品的抽提效果对比参见图2。
- **本试剂盒抽提得到的RNA纯度高**，显著优于Trizol和国外Q品牌的同类产品。本试剂盒抽提得到的RNA的A260/A280通常为2.0-2.2。A260/A230通常为1.9-2.1。本试剂盒与传统的Trizol方法及国际知名Q品牌同类产品的抽提效果对比参见图2。由图2可知，本试剂盒在抽提组织样品的RNA时，A260/A230非常显著地高于Trizol法和Q品牌的同类产品，提示纯度高。



	培养细胞			肝组织			脑组织		
浓度(ng/μl)	449	189	192	649	198	640	546	159	289
A260/A280	2.06	2.07	2.08	2.04	2.12	2.18	2.09	2.15	2.16
A260/A230	2.12	2.05	1.97	1.35	1.61	1.94	0.36	1.21	1.96

图 2. 本试剂盒与 Trizol、Q 品牌同类产品的 RNA 抽提效果对比。样品为冻存的 50 万个 HeLa 细胞、20mg 小鼠肝组织和 20mg 小鼠脑组织。洗脱液用量均为 40μl。均取 6μl 洗脱的样品经变性处理后，在含 6.67%甲醛和适量 NA-Red 的 1.2%变性琼脂糖凝胶中电泳约 45 分钟后拍照。实际抽提效果会因实验条件、检测仪器等的不同而存在差异，本图仅供参考。

注：纯的 RNA 其 A260/A280 约为 2.0，但在很多仪器上测定时会高于 2.0，低于 1.9 通常认为有蛋白、DNA 或者酚等的污染；纯的 RNA 其 A260/A230 约为 2.0 左右或者略高，低于 1.9 通常认为有碳水化合物、胍盐、多肽或酚等的污染。

- 本产品与 Qiagen 公司的 RNeasy Mini Kit 一致，用于纯化 200nt 以上的 RNA，对小于 200nt 的 small RNA 即小 RNA(如 5.8S rRNA、5S rRNA、tRNA、miRNA、piRNA 等)被选择性地去除，如果希望抽提得到小于 200nt 的小 RNA，推荐使用碧云天的 RNAeasy™动物小 RNA 抽提试剂盒(离心柱式)(R0028)或 RNAeasy™ Plus 动物 RNA 抽提试剂盒(离心柱式)(R0032)。
- 本试剂盒适用于新鲜或冻存的动物组织或细胞RNA的抽提，推荐的细胞用量为50-100万(上限可达1000万)，组织用量为15-20mg(上限可达30mg)。对于不同的组织或细胞上限会有所差别，例如小鼠肝脏组织的上限可达30mg，但肌肉组织的上限为20mg。
- 本试剂盒可用于50个样品的RNA抽提。

包装清单：

产品编号	产品名称	包装
R0026-1	裂解液	32ml
R0026-2	结合液	32ml
R0026-3	洗涤液I	32ml
R0026-4	洗涤液II	63ml
R0026-5	洗脱液	5ml
R0026-6	RNA纯化柱及废液收集管	50套
R0026-7	RNA洗脱管	50个
—	说明书	1份

保存条件：

室温保存，一年有效。

注意事项：

- 对于富含RNase的样品(如动物组织)，建议使用前在裂解液中添加二硫苏糖醇(Dithiothreitol, DTT)至终浓度为40mM (例如1ml裂解液中加入20μl 2M DTT)或β-巯基乙醇至终浓度为1% (如1ml裂解液中加入10μl β-巯基乙醇)。含DTT或β-巯基乙醇的裂解液可在室温放置1个月。
- 通过本试剂盒抽提得到的RNA已经去除绝大多数DNA，通常情况无需DNase处理。后续如进行某些对DNA残留较敏感的实验时，

可以在使用说明中步骤4离心后，在纯化柱中加入适量DNase I进行消化，具体请参考使用说明。

- 本试剂盒提供的所有试剂和耗材都是RNase-free，操作时应小心，避免被污染。所有自行准备的试剂和耗材也都应是RNase-free。如果可能有RNase污染，可考虑用0.01%的DEPC水浸泡过夜，然后高温高压灭菌并烘干。操作时应避免对着样品或所使用的试剂盒耗材呼气或说话，以防RNase污染。建议戴一次性口罩操作。
- 对于操作环境中RNA酶的去除，推荐使用碧云天生产的RNase and DNase Away (R0123)以去除实验桌面上或其它接触面上的RNase。
- 使用冻存的细胞或组织抽提 RNA 的效果通常比新鲜的细胞或组织差一些。因为在细胞或组织冻融过程中一些细胞或组织内的RNase 会被释放出来并剪切样品中的 RNA。对于组织样品，推荐使用碧云天生产的 RNALater™动物组织 RNA 稳定保存液(R0118)进行保存以保证样品 RNA 的完整性。
- 本试剂盒所有操作均在室温进行，操作时无需冰浴。所有离心也均在室温进行。
- 废液收集管在一次抽提中需多次使用，切勿中途丢弃。
- 结合液、洗涤液中含有乙醇，使用后须旋紧瓶盖以防挥发，并须按照易燃试剂的相关规范进行存放和使用。
- 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. **样品的准备**。

   细胞样品：收集50-100万左右的细胞。对于悬浮细胞，1000-2000×g离心1分钟后弃上清，加入300μl裂解液，轻轻吹打8-10次至固悬物溶解、溶液澄清；对于贴壁细胞，吸净培养液后加入300μl裂解液，轻轻吹打5-10次至固悬物溶解、溶液澄清，转移至一洁净离心管内。

   组织样品：取15-20mg动物组织，置于液氮中研磨成粉末，立即加入600μl裂解液。也可将组织置于1.5ml离心管中，迅速加入600μl冰浴预冷的裂解液，用微型电动匀浆器匀浆，或者用普通玻璃匀浆器进行匀浆。研磨或匀浆后，轻轻吹打匀浆液8-10次，室温放置3-5分钟。然后约14,000×g离心2分钟，将上清液移至新的离心管中。对于比较容易裂解且匀浆很充分的组织(如肝组织、脑组织等)可以不用高速离心而直接进入步骤2。

   注意：细胞的数量一般不超过500万，不超过100万细胞宜使用300μl裂解液，多于100万细胞宜使用600μl裂解液。动物组织的用量一般不超过30mg，用量过多可能会因裂解不充分导致得率下降。组织样品在裂解和离心后，离心管下部可能会有一些胶状物质，宜作为上清转移至下一步操作。如果弃除胶状物，会导致得率下降约30-50%。后续加入结合液后胶状物会消失。离心是为了去除未匀浆充分的明显块状物。如果裂解后仍有粘稠物，需增加吹打次数至溶液完全澄清。对于富含RNase的样品，裂解液中宜添加DTT至终浓度为40mM或添加β-巯基乙醇至终浓度为1%。
2. **加入等体积结合液至裂解液中，轻轻颠倒混匀3-5次。此时可能会有沉淀物产生，属于正常现象**。
3. **将混合物(包括沉淀物)转移至纯化柱内，12,000×g离心30秒，倒弃收集管内液体**。

   注意：当裂解液的体积大于300μl时，在加入等体积结合液后，总体积会超过纯化柱的容量，这时应分成2次过柱，即将一半的混合物过柱后，再将剩余的混合物重复步骤3一次。对于一些特殊的样品，如出现溶液未完全通过时，可延长离心时间至1-2分钟，或者加大离心力至16,000×g。对于一些能快速启动达到12,000×g的离心机，离心30秒已经足够，对于一些启动速度缓慢的离心机本步骤及后续步骤中的30秒离心宜调整为60秒。
4. **加入600μl洗涤液I，12,000×g离心30秒，倒弃收集管内液体。选做：**通过本试剂盒抽提得到的RNA已经去除绝大多数DNA，通常情况无需DNase处理。后续如进行某些对DNA残留较敏感的实验时，可在本步骤离心后，在纯化柱中加入80μl含10U DNase I的酶溶液(推荐使用碧云天RNase-free的DNase I，D7073或D7076，每80μl酶溶液按照71.8μl水加8μl 10X Reaction Buffer再加0.2μl 50U/μl DNase I混合配制而成)，室温放置消化15分钟。消化结束后，不需要进行离心等任何额外的操作，直接进入步骤5。
5. **加入600μl洗涤液II，12,000×g离心30秒，倒弃收集管内液体**。
6. **重复步骤5一次**。
7. **最高速(约14,000-16,000×g)离心2分钟，去除残留的液体**。
8. **将RNA纯化柱置于本试剂盒提供的RNA洗脱管中，加入30-50μl洗脱液，室温放置2-3分钟，最高速离心30秒，所得溶液即为纯化的RNA**。

   注意：洗脱液需要加到纯化柱柱面中央，使其被完全吸收。如需获得更高浓度的样品，可把洗脱液的体积减小至20μl，但洗脱下来的RNA量会相对减少。室温较低时，洗脱液在37°C预热片刻对得率有所帮助。此外，洗脱后的溶液再次加回到原纯化柱再离心洗脱一次，可提高得率约10-30%；或者在第一次洗脱后使用新的洗脱液再洗脱一次，会获得约为第一次洗脱量15-40%的RNA。

相关产品：

产品编号	产品名称	包装
R0011	Beyozol (总RNA抽提试剂)	100ml
R0016	Trizol (总RNA抽提试剂)	100ml
R0021	DEPC水(DNase、RNase free)	100ml
R0022	DEPC水(DNase、RNase free)	500ml
R0024	RNAeasy™动物RNA抽提试剂盒(离心柱式)	12次
R0026	RNAeasy™动物RNA抽提试剂盒(离心柱式)	50次
R0027	RNAeasy™动物RNA抽提试剂盒(离心柱式)	200次

R0028	RNAeasy™动物小RNA抽提试剂盒(离心柱式)	50次
R0032	RNAeasy™ Plus动物RNA抽提试剂盒(离心柱式)	50次
R0035	BeyoMag™磁珠法动物总RNA抽提试剂盒	50次
R0118	RNALater™动物组织RNA稳定保存液	100ml
R0121	AllProtect™动物组织核酸、蛋白稳定保存液	25ml
R0122	AllProtect™动物组织核酸、蛋白稳定保存液	100ml
R0123	RNase and DNase Away	250ml
R0125	RNase, DNase and DNA Away	250ml
R0127	RNase, DNase, RNA and DNA Away	250ml
ST036	DEPC	10g

使用本产品的文献：

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