GenePharma

DxGene™ Tissue and Cell Total RNA Extraction Kit 1

User Manual

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一．介绍

背景及原理介绍

DxGene™ 组织和细胞系总 RNA 抽提试剂盒经过一系列优化被设计用于从各种细胞和组织（包括富含脂肪的组织）样品中纯化出高质量的总 RNA，并且与 DxGene™ Total RNA Quantitation Kit, DxGene™ Her2 Test Kit, DxGene™ EGFR Test Kit，DxGene™ Beta-actin Test Kit 和 DxGene™ Her2 Duplex Test Kit 等基因检测试剂盒结合使用，为准确定量癌症患者中各指标基因包括的表达情况提供优质的总 RNA 标本。

本试剂盒将传统的酚/胍盐抽提法和硅胶膜相结合纯化总 RNA。同时试剂盒提供 Ezol™ 裂解试剂，能促进细胞和组织的裂解以及抑制 RNA 酶的活性。当总 RNA 结合到膜上时，各种污染包括酚等试剂都被洗脱，从而得到高纯度的总 RNA。总 RNA 可以用 50μl 或者更多的无 RNA 酶水溶解。

试剂盒规格

产品目录号	产品名	规格	储存条件
QPG-060	DxGene™ Tissue and Cell lineTotal RNA Extraction Kit	20 Samples	室温
QPG-061	DxGene™ Tissue and Cell lineTotal RNA Extraction Kit	50 Samples	室温

试剂盒组成

组分	规格	
	20 Samples	50 Samples
离心纯化柱	20	50
1.5ml 无 RNA 酶离心管	20	50
2.0ml 收集管	20	50
Ezol™ 裂解液	24ml	60ml
WB 缓冲液 (浓缩液)	11ml	27.5ml
无 RNA 酶洗脱缓冲液	2ml	5ml
用户手册	1	1

用户自备试剂和设备

当接触化学试剂时，请穿好实验服，戴好一次性手套和防护目镜。

试剂类	氯仿 95%~100%乙醇。 无 RNA 酶水/DEPC 水
设备和耗材类	无 RNA 酶，灭菌的枪头。 一次性手套 可用来进行组织破碎和匀浆的设备和离心管 无 RNA 酶的 1.5ml 和 2ml 离心管 能进行室温和 4℃离心的台式离心机（附带 2ml 离心管的转子）。

相关产品

我们也同时提供 DxGene™ Total RNA Quantitation Kit, DxGene™ Her2 Test Kit,DxGene™ EGFR Test Kit，DxGene™ Beta-actin Test Kit 和 DxGene™ Her2 Duplex Test Kit 等配套使用的试剂盒，详情请联系我们。

目录号	名称	规格
QPG-070	DxGene™ Total RNA Quantitation Kit	20 rxns
QPG-080	DxGene™ Her2 Test Kit	100rxns
QPG-081	DxGene™ EGFR Test Kit	100rxns
QPG-082	DxGene™ Beta-actin Test Kit	100rxns
QPG-083	DxGene™ Her2 Duplex Test Kit	100rxns

二．操作步骤

样品类型需求

DxGene™ 组织和细胞系总 RNA 抽提试剂盒是被开发和优化用来从新鲜组织和细胞中提取总 RNA。至于石蜡包被样品中总 RNA 的提取，请选择 DxGene™ FFPE Total RNA Extraction Kit。

实验流程

1. 从组织中提取总 RNA，在冷冻的研钵中切下一小块深层冷冻的组织样品，并用电子天平称量。然后立即将该组织转移至加有 1 ml Ezol 裂解液的无 RNA 酶的离心管中，用电动匀浆机在冰浴中匀浆组织约 15s 直到看不见明显的组织块，之后操作见第 3 步。

   注意：组织样品应储存于液氮或低温冰箱 (-80^{o}C)。该实验流程只适用于在小于 50mg 的组织样品中抽提总 RNA，如果样品比较多，则需要适量多加 Ezol 裂解液来消化组织。

2. 从细胞样品中提取 RNA，先把培养皿或培养瓶置于冰上，弃尽培液，用预冷的 1XPBS 洗涤细胞两次。然后加入 1 ml Ezol 裂解液，用细胞刮刮下所有细胞，将裂解物转移至无 RNA 酶的 1.5ml 离心管中，充分振荡 30s，之后操作见第 3 步。

   注意：

   1. 试剂盒中不提供 1XPBS，可另外索取。

   2. 重悬细胞还可以先将细胞 2000Xg，离心 3min，用 1XPBS 洗涤后再加入 1ml Ezol 裂解液。

   3. 该实验流程只适用于从少于 1 x 10^{7} 细胞中提取 RNA，否则就需要更多的 Ezol 裂解液。

3. 把裂解物于室温静置 5min 后，加入 0.2ml 三氯甲烷，小心盖上管盖，剧烈摇动 15s。

   注意：请不要漩涡震荡，避免引入 DNA 污染。

4. 将离心管再次置于室温 2-3 min，然后 4℃，12,000 x *g* 离心 15 min。
   注意：离心会使溶液分为三相，含有 RNA 的水相上清液，包含蛋白和 DNA 的中层以及包含 DNA 的下层有机相。在 4℃离心对于溶液的分层是非常关键的。

5. 将上清水相转移至另一新的无RNA酶离心管中，并加入等体积的 70%乙醇，漩涡振荡混匀 5s。
   注意：如果不需要抽提 DNA，请弃尽中层和下层有机相。

6. 立即吸取 700μl 样品以及有可能形成的沉淀，加入带有 2ml 收集管的 mini-spin 离心柱。轻盖盖子， 8,000 x *g*，室温离心 15 s，弃尽流穿液。

7. 将剩余的样品转移至离心柱，重复第 6 步。

8. 往离心柱中加入 700μl WB，轻盖盖子，8,000 x *g*，室温离心 15 s，弃尽流穿液。
   注意：第一次使用前请确认是否已经往 WB 中加入适量乙醇。

9. 重复第 8 步，用 500μl WB 洗涤离心柱两次，最后一次洗涤后，8,000 x *g* 离心空柱 2 min 干燥硅胶膜。
   注意：完全去除洗液对最后溶解是非常重要的，洗液的残留会影响最终的洗脱。

10. 将离心柱转移至一新的无 RNA 酶的 1.5ml 离心管中，往硅胶膜中央滴加 40μl 无核酸酶水，轻盖管盖，室温静置 1-5 min，8,000 x g 离心 1 min 洗脱 RNA。

11. 把洗脱液滴加回硅胶膜重复第 10 步洗脱。

12. 始终将溶解的 RNA 至于冰上，然后于-70℃保存。

三．疑难问题处理

本试剂盒经过精心的包装并含有高质量的试剂，这部分疑难问题处理主要提供了针对组织样品操作问题的常规指导。也许您还会遇到其他的问题，届时我们也非常欢迎您来和我们的技术服务人员进行探讨。

液相没有完全分开

混匀和匀浆不充分

通常此类问题是由于加了氯仿之后不充分的混匀以及随后在比较高的温度下离心引起。正如实验操作流程中第 3 和第 4 步所述，在加入氯仿后必须剧烈摇动混匀至少 15s，并且随后在 4℃或者不高于 8℃的温度下离心，较高的离心温度会破坏溶液分层。

纯化柱堵塞

匀浆不充分

不充分的匀浆可能导致大块组织残留堵塞纯化柱，必要时请增加离心场和离心时间。

起始样品太多

起始组织样品不宜超过 100mg，必要时请减少样品用量。

RNA 降解

样品或素材保存环境太差

状态不好，或者经过某些药物处理的细胞或组织中的 RNA 有可能会发生自发的降解，请将标本存放于-80℃或者液氮中。如果能加入一些商业化的 RNA 酶抑制剂（如 RNA Later），效果会更好。

样品采集

在采集到组织标本后尽快的使 RNA 酶丧失活性对防止 RNA 降解是至关重要的。另外，请尽量缩短处死到取样的时间，这样才能保证高纯度 RNA 的高得率。

裂解前样品操作

在把组织样品投入裂解液前，请保持冷冻的状态，在样品解冻之前，尽快将其投入含 RNA 酶抑制剂的裂解液中，然后立即匀浆。

离心条件

加入氯仿之后的离心请务必在 4℃。

电泳图显示降解

如果在电泳时总 RNA 上样超过 5μg，单一泳道中的总 RNA 条带就会出现弥散和拖尾现象。一般电泳时总 RNA 的上样量为 700ng 至 800ng 左右。

外源 RNA 酶的污染

在抽提总 RNA 的过程中，务必防止外源核酸酶的污染，整个过程始终需戴手套，并且经常更换以防止“手指残留 RNA 酶”污染。研钵和研杵必须在 180℃下烘烤过夜，以除去残留的 RNA 酶。所有的离心管和吸头必须用 DEPC 水浸泡并高压灭菌后方可使用。

RNA 降解是由于核糖核酸酶（RNase）造成，这是一种非常稳定和高活性的酶，尽管试剂盒中提供的耗材和试剂都是无 RNA 酶的，但是 RNA 酶会存在于整个工作环境中，比如双手，实验工作台和其他设备，因此很显然，RNA 样品可能在抽提中或者之后的保存过程中污染到 RNA 酶，所以我们提出如下预防措施：**1).** 请使用无 RNA 酶和一次性的用品。**2).** 用含 RNA 酶抑制剂的溶液来清洁工作台面以及相关设备。**3).** 操作试剂和 RNA 时请带上乳胶手套。**4).** 尽快的采集组织样品，避免组织样品接触到任何表面。**5).** 在进行下游操作分析时，请始终把 RNA 样品置于冰上。**6).** 将 RNA 样品分装后储存于-70℃，减少操作中反复冻融。**7).** 使用去垢剂溶液清洁电泳槽，在电泳时请用 DEPC 水防止 RNA 降解。**8).** 请用试剂盒中提供的无 RNA 酶水稀释 RNA 样品。

低得率

RNA 的得率的变化是非常普遍的现象，但是 RNA 极端的低得率可能由于抽提的失败而导致。很多因素会降低 RNA 的得率，例如低质量的组织样品，不充分的匀浆以及低效的洗脱。因此，选取高质量的组织样品并进行合理的操作是非常重要的，在 Ezol 中充分的匀浆组织对于 RNA 的获得也是必要的，另外在一些情况下，RNA 的低得率是因为低效的洗脱，适当的延长洗脱时的孵育溶解时间有助于提高 RNA 的得率。

A_{260}/A_{280} 比值偏低

通常，A_{260}/A_{280} 比值偏低是由于在水中测量吸收值，或者是由于酚和其他有机药品的污染造成。在一般情况下，分离出的 RNA 应该不含酚和其他有机药品。另外，不充分的匀浆可能导致蛋白和核酸的共纯化，从而使 280nm 处的吸收值增大。我们推荐您按照手册中的标准流程消化和匀浆组织样品，另一方面，我们也推荐您在 10mM 的 Tris-HCl 溶液中测量 RNA 的 A_{260}/A_{280} 比值。

四．后续应用

应用

抽提纯化得到的总 RNA 能应用于多种您所需要的后续实验，如 northern blotting 和 Real-time PCR，同时高纯度高质量的总 RNA 能给您提供更加稳定放心的实验结果，除此之外，本试剂盒还能保留小片段 RNA，如 microRNA，也可提供您微小 RNA 的研究使用。

相关产品和服务

为了得到稳定的基因检测结果，本试剂盒同时和 DxGene™ Total RNA Quantitation Kit, DxGene™ Her2 Test Kit, DxGene™ EGFR Test Kit，DxGene™ Beta-actin Test Kit 和 DxGene™ Her2 Duplex Test Kit 共同出售，可以为研究者提供从抽提，定量到检测一整套便利的服务。吉玛的技术人员总是乐意回答您所遇到的任何问题，无论是操作步骤还是技术上的问题，我们都将给与最大的支持。请看我们的附录来获取更多联系信息。

五．附录

总 RNA 储存

RNA 应溶解于无 RNA 酶的水里，一般没有必要额外添加其他试剂，如 RNA 酶抑制剂或者 RNA 稳定剂。洗脱的 RNA 可以储存于-70℃或者-20℃，如果长期保存，我们推荐在-70℃。在这种低温环境下，3 年后也并未观察到 RNA 有显著的降解。为了防止由于反复冻融而造成的 RNA 降解，请分装后储存。

总 RNA 定量

本试剂盒所抽提的RNA基本上没有任何有机药品的污染，因此非常适合用紫外分光光度计进行定量。RNA的浓度可以在一个石英或者紫外适用的比色皿中通过测量 260nm处的吸收值来计算得到，260nm处一个单位的吸收值等于 40 μg/ml的RNA，这种计算关系只适用于在水中进行定量分析，因此RNA必须用无RNA酶水溶解，稀释和测量，同时为了最大限度减少差异，样品的吸收值应控制在 0.1-1.0 之间。然而，由于抽提得到RNA的量较少，因此我们推荐使用超微量的比色皿，只需要消耗常规比色皿的十分之一的RNA就能进行测量。适用于紫外测量的比色皿您可以从几个生物公司购得：例如BrandTech Scientific (www.brandtech.com)和Santa Cruz Biotechnology (www.scbt.com)。在测量之前，我们推荐先用RNA标准溶液校正紫外分光光度计，RNA标准溶液可另行购买。

实例：用常规比色皿测量 RNA 浓度。

RNA 样品总体积：50 μl

稀释因子，1:100（10 μl RNA 样品+990 μl 无 RNA 酶高压灭菌水）

A_{260} 读值：0.20（在 1ml 比色皿中测量）

因此，RNA 样品浓度= 40×A_{260}×稀释因子=40×0.20×100=800 μg/ml

RNA 样品的得率=800×0.05=40 μg

实例：用超微量比色皿测量 RNA 浓度。

RNA 样品总体积：50 μl

稀释因子，1:100（1 μl RNA 样品+99 μl 无 RNA 酶高压灭菌水）

A_{260} 读值：0.20（在超微比色皿中测量）

因此，RNA 样品浓度= 40×A_{260}×稀释因子=40×0.20×100=800 μg/ml

RNA 样品的得率=800×0.05=40 μg

用户可以选择其他方法，比如 RiboGreen 法（Invitrogen）或者 NanoDrop 设备（Thermo Scientific）来测量本试剂盒抽提出来的 RNA 的浓度，应该能获得和紫外分光光度计一致的结果。但是用户们必须于测量样品之前在 RiboGreen 法中先建立一条标准曲线，或者先用 RNA 标准品校正 Nanodrop。如果用户采用其他方法来测量 RNA 浓度，我们建议用户严格按照该厂商的使用手册进行操作。

总 RNA 质量的判定

完整性

本试剂盒提取的 RNA 的大小和完整性可以用变性胶电泳或者 Bioanalyzer 来鉴定。通常经过溴乙锭染色后，胶上会呈现出两条锐利的条带，它们分别是 18s 和 28s 核糖体 RNA。一个具有良好完整性的 RNA 样品，28s 和 18s 的强度比应接近 2:1，Bioanalyzer 能提供更准确的完整性信息。

注意：由于配套的 DxGene™ 荧光定量检测试剂盒是设计用来扩增短于 100 个核苷酸的 mRNA 片段，因此即使完整性有少许不合格的样品也会给最终的检测结果造成细微的影响。

纯度

本试剂盒将传统的胍盐/酚/氯仿法和纯化柱相整合，在试剂盒所述的步骤和环境下能分离出高质量的 RNA，因此，我们不推荐您检测 RNA 的纯度，然而由于某些原因，用户想证明其纯度，这样的话用 A_{260}/A_{280} 的吸收比值可以对 RNA 的纯度作大概评价。通常来说，高纯度 RNA 样品的 A_{260}/A_{280} 应在 1.9~2.1 之间，然而该吸收比值容易受 PH 变化的影响，因为水不是缓冲体系，因此在水中测量的 A_{260}/A_{280} 会略低于在缓冲液中测量的结果，并且结果之间会有很大的差异，结果可能导致一些误解，例如样品是否被蛋白，酚或者其他有机试剂污染，因为这些污染都在 280nm 处有高吸收值。所以，为了结果的准确性，我们推荐您在 10 mM Tri-HCl (pH 7.5) 的缓冲体系中测量，并用相同的缓冲液校准紫外分光光度计。

注意：

1. ***RNA 浓度必须在水中测量，因为吸收值和 RNA 浓度之间的换算关系取决于 RNA 在水中的消光系数。***
2. ***本试剂盒已经过优化来防止 DNA 污染，我们的测试结果表明不需要再额外添加 DNA 酶。用本试剂盒分离的 RNA 完全适用于配套的 DxGene™ 荧光定量检测试剂盒，其中引物和探针都是针对目标 mRNA 特异设计的。同时也请您在检测实验时引入“no RT”对照来监控 DNA 可能的干扰。***

技术服务

万维网

您可以用您的网页浏览器访问我们的网站，您可以得到以下信息：

- □ 查找相应产品的货号
- □ 了解我们的新产品
- □ 咨询产品和相应的文章

吉玛网址www.genepharma.com

质量控制

我们有严格的质量控制体系，每一批产品都经过MCF7和MDA-MB-231两种细胞系的总RNA进行过功能性测试。

联系我们

如果您想获取更多的的技术支持，请致电，写信，电子邮件或是传真。

公司总部：
中国上海张江高科技园区哈雷路1011号
电话：86-21-51320195
传真：86-21-51320295
电子邮件：service@genepharma.com

I. Overview

Introduction and Principle

DxGene™ Tissue and Cell line Total RNA Extraction Kit is developed and optimized for total RNA of high quality extraction from cell lines and tissue samples including lipid tissue samples.It can be used with DxGene™ Total RNA Quantitation Kit,DxGene™ Her2 Test Kit, DxGene™ EGFR Test Kit, DxGene™ Beta-actin Test Kit and DxGene™ Her2 Duplex Test Kit, providing total RNA of high quality for detecting and quatitation of Her2 and EGFR gene accurately in cell and tissue samples

DxGene™ Tissue and Cell line Total RNA Extraction Kit integrates traditional phenol/guanidine-based lysis and silica-gel-membrane purification of total RNA.The kit also provides Ezol™ lysis reagent,designed to facilitate lysis of samples and inhibit RNase.After RNA binding to the silica menbrane, phenol and other contaminations are efficiently washed away by the WB buffer in the kit.Then high quality of total RNA is eluted in 50μl or more RNase-free water.

Kit Specification

Cat. No.	Item	Specificaion	Storage
QPG-060	DxGene™ Tissue and Cell lineTotal RNA Extraction Kit	20 Samples	RT
QPG-061	DxGene™ Tissue and Cell lineTotal RNA Extraction Kit	50 Samples	RT

Kit Contents

Contents	Specification	
	20 Samples	50 Samples
Mini-spin Column	20	50
1.5ml RNase-free tube	20	50
2.0ml Collection tube	20	50
Ezol™ Reagent	24ml	60ml
WB Buffer (Concentrate)	11ml	27.5ml
RNase-free Eution Buffer	2ml	5ml
User Manual	1	1

Equipment and Reagents to Be Supplied by User

When working with chemicals,always wear a lab coat,disposable gloves and protective goggles.

Reagent

Chloroform
95%~100% Ethonal
RNase-free water/DEPC water

Equipment

Sterile,RNase-free tips
Disposable gloves
Eqiupment and tubes for tissue disruption and homogenization.
RNase-free 1.5ml or 2ml microcentrifuge tubes
Microcentrifuges (with a rotor for 2ml tubes) for centrifugation at 4℃ and room temperature.

Related Products

DxGene™ Total RNA Quantitation Kit, DxGene™ Her2 Test Kit, DxGene™ EGFR Test Kit, DxGene™ Beta-actin Test Kit and DxGene™ Her2 Duplex Test Kit are also provided by GenePharma.You can contact us for more information.

Cat. No.	Item	Specification
QPG-070	DxGene™ Total RNA Quantitation Kit	20 rxns
QPG-080	DxGene™ Her2 Test Kit	100rxns
QPG-081	DxGene™ EGFR Test Kit	100rxns
QPG-082	DxGene™ Beta-actin Test Kit	100rxns
QPG-083	DxGene™ Her2 Duplex Test Kit	100rxns

II. Protocol

Sample type requirement

DxGene™ Tissue and Cell line Total RNA Extraction Kit is developed and optimized for total RNA isolation in fresh tissue samples or cell samples.As to RNA extraction in FFPE samples,please select DxGene™ FFPE Total RNA Extraction Kit.

Protocol

1. To extract RNA from tissues, cut a piece of deep-frozen tissue in a chilled mortar, and weight by electronic balance. Transfer tissue immediately into a 1.5-ml RNase-free tube which already contains 1 ml of Ezol reagent. Homogenize tissue with an electronic homogenizer on ice for 15 s or until no visible tissue, and then move to step 3.

   Note: Tissue sample should be stored in liquid nitrogen or in deep freezer (-80^{o}C). This protocol is suitable for isolation of RNA from less than 50 mg of tissue; otherwise, more Ezol reagent is needed to digest tissue sample.

2. To extract RNA from cells, put cell culture dish or flask on ice, remove culture medium, and wash cells twice with ice-cold 1X PBS. Add 1 ml of Ezol reagent and scrape cells off the plate with cell lifter. Transfer cell lysates into 1.5-ml RNase-free tube, vertex thoroughly for 30 s, and then move to step 3.

   Note: PBS buffer is not provided with kit, and is available separately.

   Note: For suspension cells, spin down cells first at 2,000 x g for 3 min, wash cells twice with 1X PBS, and then add 1 ml of Ezol reagent.

Note: This protocol is suitable for isolation of RNA from ≤1 x 10^7 cells, or from a ≤ 10-cm diameter dish; otherwise, more Ezol reagent is needed to homogenize cells.

3. Incubate mixture at room temperature for 5 min, and add 0.2 ml of chloroform. Cap the tube securely, and shake it vigorously for 15 s.
   Note: Avoid vertexing as this may increase the DNA contamination to the RNA sample.

4. Place the tube at room temperature for another 2-3 min, and centrifuge at 12,000 x *g* for 15 min at 4 ^{o}C.
   Note: Centrifugation separates solution into three phases: upper aqueous with RNA, middle layer with protein/DNA, bottom organic with DNA. The 4 ^{o}C is essential for phase separation.

5. Transfer the upper aqueous phase to a new 1.5-ml sterile tube, and add 1 volume of 70% ethanol. Mix solution thoroughly by vortex for 5 s.

   Note: Discard the middle layer and bottom organic phase if no need for isolation of DNA.

6. Pipet up to 700 µl of the sample immediately, including any precipitate that may have formed, into a mini-spin column in a 2-ml collection tube. Close tube gently, and centrifuge at 8,000 x *g* for of 15 s at room temperature.Discard the flow-through.

7. Repeat step 6 using the remaining sample, and discard the flow-through.

8. Add 700 µl of Wash Buffer (WB) into a mini-spin column. Close the tube gently, and centrifuge at 8,000 x *g* for 15 s. Discard the flow-through.

   Note: Ensure that three volume of 100% ethanol is added to WB before using for the first time.

9. Repeat step 8 twice with 500 µl wash buffer. After last wash, centrifuge column for 2 min at 8,000 x *g* to dry silica-gel membrane.

   Note: It is important to completely remove washing solution. Any carryover may affect elution.

10. Transfer column into a new RNase/DNase-free 1.5-ml tube, pipet 40 µl RNase/DNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, wait 1 to 5 min, and centrifuge for 1 min at 8,000 x *g* to elute RNA.

11. Load elute back to the column, and repeat the elution step 10.

12. Keep eluted RNA sample on ice all the time and store at -70^{o}C.

III. Troubleshooting

Kit is carefully assembled and contains high quality of reagents. This troubleshooting is to provide a general guide for solving the problems which are often related to tissue handling and operation. Many other problems may occur during extraction.You are always welcome to discuss any problem with our technique service.

Phases don't separate completely

Insufficient homogenate and mix	This is usually caused by insufficient mix after addition of chloroform, or by centrifugation at a high temperature. As described in step 3 and 4 of the protocol, you need to shake the tube vigorously for at least 15 s after addition of chloroform. Centrifuge the tube at 4^{o}C or no higher than 8^{o}C. A higher temperature will disrupt phase separation.

Clogged Column

Insufficient homogenate	Insufficient homogenate may result in large tissue piece that may clog the column.Please increase the g-force and time of centrifugation if necessary.
Too much starting material	The starting material can not be more than 100mg tissue.Please reduce the amount of starting material if necessary.

Degradation of RNA

Sample or material stored in bad condition	Total RNA in cells or tissues that in bad condition or dealed with some medication may lead to self-degradation.Please store samples in -80C or liquid nitrogen.It should be better if some commercial RNase inhibitor such as RNA Later.
Sample Collection	It is important to inactivate RNase as soon as possible once samples are gathered.Please shorten the time of collection as possible as you can to yield higher quality of total RNA.

Sample manipulation before lysis	Keep tissue frozen before lysis.Please transfer tissue sample into lysis buffer contains RNase inhibitor before thawing and homogenize it in ice bath immediately.
Centrifugation	Please centrifuge samples at 4C after adding chloroform.

Degradation after electrophoresis	If loading RNA is more than 5μg, the band will show some smear.So please take about 700ng to 800ng total RNA for electrophoresis.
Exogenous RNase contamination	Disposable gloves and respirator must be used during the whole process to prevent any RNase contamination.The mortar and muller should be dried and heated at 180℃ over night before use to eliminate RNase.All the tubes and tips needed should be treated by DEPC water and then autoclave them before use.

RNA is degraded by ribonucleases (RNases) which are very stable and active enzymes. Although the materials and reagents provided by kit are free of RNases, RNases may be present in the working environment, such as on hands, laboratory bench, and equipment. Therefore, it is perceivable that RNA samples could be contaminated by RNases during or after isolation procedure. We suggest you take following precautions to avoid inadvertent RNase contamination. **1**) Use RNase-free and disposable supplies; **2**) Clean up the bench top or equipment with RNase inhibitor-containing solution; **3**) Always wear latex or vinyl gloves while handing reagents and RNA samples; **4**) Collect tissue samples as quickly as possible and avoid contact with any surface; **5**) Keep purified RNA samples on ice while preparing for downstream analysis; **6**) Store RNA sample at -70^{o}C in aliquot to reduce freeze/thaw cycle; **7**) Clean electrophoresis tank with detergent solution (e.g., 0.5% SDS) and use DEPC-treated water to prevent RNA degradation in gel running; **8**) Always dilute RNA samples with RNase/DNase-free water provided with kit.

Low Yield	It is normal to see the variation of RNA yield. However, an extreme low yield of RNA may indicate failure of extraction. Many factors can reduce the yield of RNA, such as poor quality of tissue samples, insufficient homogenization, and poor elution. It is important to select good quality tissue and handle it appropriately. Complete homogenization in Ezol reagent is also essential for extraction of RNA. In some

	cases, a low yield of RNA is caused by poor elution, and an extended incubation with RNase-free water on column may enhance RNA recovery.
Low A_{260}/A_{280} Value	Usually, a low A_{260}/A_{280} value is caused by measurement of absorbance in water, or by contamination of proteins, phenol, or other organic chemicals. Under normal conditions, the isolated RNA should be free of phenol and organic chemicals. However, insufficient homogenization may cause co-purification of proteins and increase reading at 280 nm. We recommend following the described procedures to digest and homogenize tissue samples. We also recommend measuring the ratio of A_{260}/A_{280} in 10 mM Tris-HCl buffer.

IV. Downstream Application

Application	Isolated total RNA can be applied for many kinds of experiments such as real-time PCR, northern blotting or genechip that you need.The high purity and quantity RNA extracted may provide you more consistent result.Besides,this kit also retain small RNA including microRNA that you can also detect for research.
Related products and Service	Also,for getting consistent results for gene detection,this kit is sold paired with DxGene™ Total RNA Quantitation kit,DxGene™ Her2 Testing Kit,DxGene™ EGFR Testing Kit and DxGene™ Duplex Testing Kit.It is convenient for researcher to deal with samples from extraction,quatitation to the detection.The technical support in Genepharma is always happy to answer any questions you may have either the information or the protocol in this manual and other related products.Please see the appendix for more contact information.

V. Appendix

Storage of RNA Samples

RNA is eluted in RNase/DNase-free water. There is no need to add any other reagents, such as RNase inhibitors or stabilizers. The eluted RNA samples can be stored at -20 or -70°C. Storage at -70°C is recommended for long-term usage. Under this condition, no obvious degradation of RNA is detectable after 3 years. To avoid possible degradation caused by frequent freeze/thaw, samples may be aliquoted before storage.

Quantitation of RNA

RNA samples prepared by this kit are basically free of contamination by any organicchemicals, and therefore, are well suitable for quantitation by spectrophotometer method. The concentration of RNA can be determined by measuring the absorbance at 260 nm (A_{260}) using quartz or UV-compatible cuvettes. An absorbance of 1 unit at 260 nm is equivalent to 40 μg of RNA per ml. This relationship is valid only in water, so RNA samples should be diluted or measured in RNase/DNase-free water. To minimize variation, readings of sample absorbance should be between 0.1 and 1.0. Given that the isolated RNA is usually in small quantity, we recommend using ultra-micro cuvettes, which consume only one tenth of RNA sample required for measurement in regular cuvettes. UV compatible ultra-micro cuvettes are available from several biotech companies, such as BrandTech Scientific (www.brandtech.com) and Santa Cruz Biotechnology (www.scbt.com). Before measuring samples, we also recommend calibrating spectrophotometer with RNA standard solution, which is available separately.

Note: An example of determination of RNA concentration using regular cuvette:

Total volume of RNA sample, 50 μl
Dilution Factor, 1:100 (10 μl of RNA sample + 990 μl of sterile water)
A_{260} Reading, 0.20 (measured in a 1-ml cuvette)
So, Concentration of RNA sample = 40 x A_{260} x dilution factor
= 40 x 0.20 x 100 = 800 μg/ml
And, Total yield of RNA sample = 800 x 0.05 = 40 μg

Determination of RNA concentration for the same sample using ultra-micro cuvette:

Total volume of RNA sample, 50 μl

Dilution Factor, 1:100 (1 μl of RNA sample + 99 μl of sterile water)

A_{260} Reading, 0.20 (measured in an ultra-micro cuvette)

So, Concentration of RNA sample = 40 x A_{260} x dilution factor

= 40 x 0.20 x 100 = 800 μg/ml

And, Total yield of RNA sample = 800 x 0.05 = 40 μg

Customers may also use other methods, such as RiboGreen (Invitrogen) or NanoDrop equipment (Thermo Scientific), to determine RNA concentration for the samples prepared by this kit, and should obtain comparable results as spectrophotometer described above. However, the customers need to prepare a RNA standard curve in RiboGreen assay, or to calibrate NanoDrop machine by a RNA standard before measuring samples. Customers are also encouraged to follow manufactory's manual if other methods are used for quantitation of RNA samples.

Judgement of the Qaulity of Total RNA

Integrity

The integrity or the size of total RNA purified by this kit can be checked by denaturing agarose gel electrophoresis, or by a Bioanalyzer. Normally, there will be two sharp bands on gel following ethidium bromide staining, which represent 18S rRNA and 28S rRNA. If a sample shows good integrity of RNA, the ratio of the intensity of 28S and 18S will be close to 2:1. Bioanalyzer can provide more accurate analysis of RNA integrity.

Note: Given that GeneMedDx™ real-time PCR kits are designed to amplify the mRNA fragments of shorter than 100 nucleotides, a compromised RNA integrity is expected to have marginal impact on the real-time PCR assay.

Purity

This kit has integrated classic guanidine/phenol/chloroform extraction method and column purification process, and should produce high purity samples under the conditions described in the kit. Therefore, we do not recommend testing the purity. If, for some reasons, customers need to document sample purity, the ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) can be measured to provide an estimate of the purity of RNA.

Generally speaking, a pure RNA sample has an A_{260}/A_{280} ratio of 1.9-2.1. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffed, the A_{260}/A_{280} ratio measured in water is often lower than that in buffer solution, and may vary greatly. As a consequence, the sample may be misinterpreted to have been contaminated with protein, phenol or other organic chemicals which usually have high absorption at 280 nm. For accuracy, we recommend measuring absorbance in 10 mM Tri-HCl (pH 7.5), and be sure to calibrate spectrophotometer with the same buffer.

Note: RNA concentration should be determined in water, since the relationship between absorbance and concentration is based on an extinction coefficient calculated for RNA in water.

Note: This kit is optimized to prevent DNA contamination. Our tests indicate that there is no need to treat the samples with DNase. RNA samples prepared by this kit are fully compatible with GeneMedDx™ real-time PCR kits, which are designed to have the primers and probes specific for amplification of mRNA molecules, and also to contain 'No RT' control in the assay for monitoring possible interference by DNA.

Technical Service

World Wide Web

Visit the GenePharma Web Resource using your World Wide Web browser. At the site, you can:

- □ Explore our catalog with full color graphics
- □ Get the scoop on our hot new products and special product offers
- □ Request catalog and product literature

The Genepharma URL is **www.genepharma.com**

Quality Control

The product has good QC system and is tested functionally by qRT-PCR using both MCF7 and MDA-MB-231 total RNA as template for every batch.

Contact Us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.genepharma.com).

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