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Not for use in diagnostic procedures.

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High Pure RNA Tissue Kit

Version: 11
Content version: September 2017

For isolation of total RNA from tissue

Cat. No. 12 033 674 001 1 kit
50 isolations

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	green	Lysis / Binding Buffer	Contains 4,5 M guanidine-HCl, 100 mM sodium phosphate pH 6.6 (25°C)	▪ 25 ml
2	white	DNase I	Recombinant lyophilizate for digesting residual contaminating DNA	▪ 10 kU DNase I
3	white	DNase Incubation Buffer	Contains 1 M NaCl, 20 mM Tris-HCl, 10 mM MnCl2, pH 7.0 (25°C)	▪ 10 ml
4	black	Wash Buffer I	Contains 5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 (25°C) (final concentration after addition of ethanol)	▪ 33 ml, add 20 ml absolute ethanol
5	blue	Wash Buffer II	Contains 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (25°C) (final concentrations after addition of ethanol)	▪ 10 ml, add 40 ml absolute ethanol
6	colorless	Elution Buffer	Water, PCR Grade	▪ 30 ml
7		High Pure Filter Tubes	For use of up to 700 µl sample volume.	▪ One bag with 50 polypropylene tubes with two layers of glass fiber fleece
8		Collection Tubes		▪ One bag with 50 polypropylene tubes (2 ml).

⚠ ***All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.***

i The buffers can show a slight yellow color. This will have no impact on the function of the buffer.

1.2. Storage and Stability

Storage Conditions (Product)

⚠ ***The High Pure RNA Tissue Kit components must be stored at +15 to +25°C. If properly stored, all kit components are stable until the expiration date printed on the label.***

⚠ ***Improper storage at +2 to +8°C (refrigerator) or −15 to −25°C (freezer) may lead to formation of salt precipitates in the buffers which will adversely impact the performance of the kit.***

Storage Conditions (Working Solution)

Solution	Storage
DNase I	−15 to −25°C

1.3. Additional Equipment and Reagents Required

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 × g centrifugal force (*e.g.,* Eppendorf 5415C or equivalent)
- Microcentrifuge tubes,1.5 ml, sterile
- Mortar and pestle or Rotor-Stator Homogenizer (*e.g.,* Ultra Turrax)

1.4. Application

The High Pure RNA Tissue Kit is designed to purify total, intact total RNA from tissue samples (e.g., mouse liver, spleen, lung, heart) for use in research. The isolated RNA may be used directly as template for RT-PCR, northern blotting, and RNAse protecion assays.

1.5. Preparation Time

Total time	Approx. 30 minutes (with rotor-stator homogenization) Add additional time when using alternative disruption methods.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Solid tissue (e.g., mouse liver, spleen, lung, heart), 1 to 10 mg (for mortar / pestle disruption) or 1 to 25 mg (for rotor-stator homogenization).

General Considerations

Handling Instructions

RNA in sample material is subject to degradation by intracellular RNases. It is therefore imperative that
- samples are immediately flash frozen in liquid nitrogen and stored at −70°C or are processed as soon as collected.
- frozen tissue should not be allowed to thaw during handling (e.g., weighing).
- the relevant procedures should be carried out as quickly as possible.

Samples can also be stored at −60°C in Lysis / Binding Buffer after disruption and homogenization.

Yields may vary depending on storage time

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Guanidine-hydrochloride in Lysis/Binding Buffer and Wash Buffer I is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.

Do not allow these buffers to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping it up.

Never store or use the Binding Buffer near human or animal food.

Always wear gloves and follow standard safety precautions when handling these buffers.

Use sterile disposable polypropylene tubes and tips to avoid RNase contamination. Always wear gloves during the assay.

Do not allow the Lysis/Binding or Wash buffers to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR setup and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

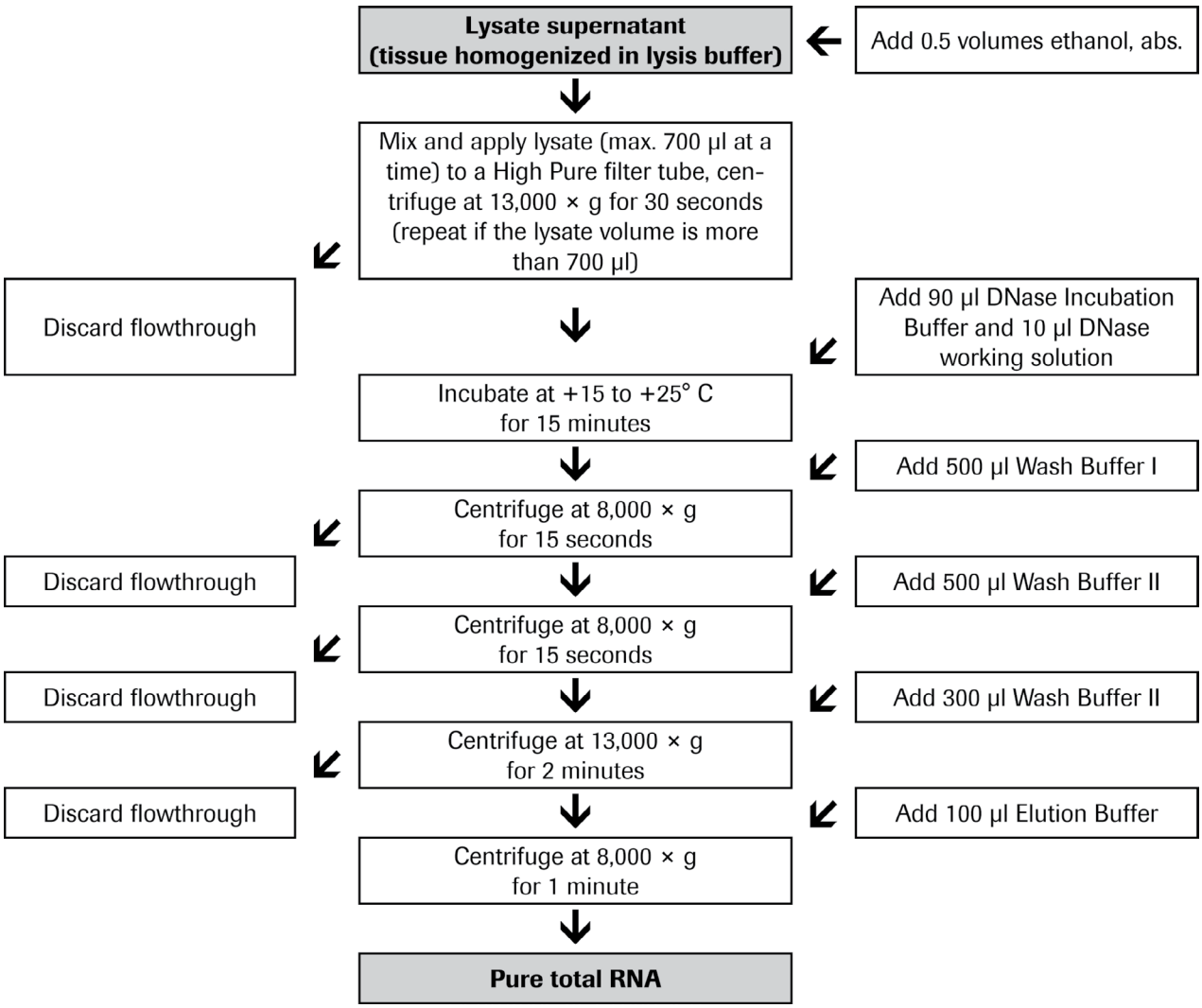
Working Solution

In addition to the ready-to-use solutions supplied with this kit, prepare the following working solutions:

Content	Reconstitution / Preparation	Storage / Stability	For use in...
DNase I (Vial 2; white cap)	Dissolve DNase I in 550 µl Elution Buffer and mix thouroghly.	▪ Store aliquots at −15 to −25°C ▪ Stable for 12 months.	Purifies the RNA from residual genomic DNA, step 7
Wash Buffer I (Vial 4; black cap)	Add 20 ml absolute ethanol to Wash Buffer. ⚠ ***Label and date bottle accordingly after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable until expiration date printed on kit label.	Removes inhibitors, step 8
Wash Buffer II (Vial 5; blue cap)	Add 40 ml absolute ethanol to Wash Buffer. ⚠ ***Label and date bottle accordingly after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable until expiration date printed on kit label.	Purifies the template DNA from residual impurities, step 9 and 10

2.2. Protocols

Experimental overview



Isolation Protocol

Disruption and Homogenization

- Efficient disruption and homogenization of the starting material is essential for intra-cellular RNA isolation procedures from tissues.
- The full disruption of cell walls and plasma membranes of cells and organelles is absolutely required to release all the RNA contained in the sample. Incomplete disruption results in significantly reduced yields.
- Homogenization is necessary to reduce the viscosity of the cell lysates produced by disruption. Homogenization shears the high molecular weight genomic DNA and other high molecular weight cellular components to create a homogeneous lysate.
- Incomplete homogenization results in significantly reduced yields. Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step.

Disruption and Homogenization using Rotor-Stator Homogenizers

In the presence of lysis buffer, rotor-stator homogenizers thoroughly disrupt and simultaneously homogenize tissues in 5 to 90 seconds, depending on the viscosity of the sample. By a combination of turbulence and mechanical shearing, the sample will be disrupted and homogenized. Keep foaming of the sample to a minimum by keeping the tip of the homogenizer submerged and holding the immersed tip to one side of the tube.

Disruption using a Mortar and Pestle

⚠ ***This step is for disruption only. Homogenization must be performed separately!***

For disruption using a mortar and pestle, freeze the sample immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the frozen tissue powder into a tube with 350 µl Lysis / Binding Buffer. Continue as quickly as possible with the homogenization step.

Homogenization using a Syringe and Needle

After disruption, the tissue lysate can be homogenized using a syringe and needle. High molecular weight DNA is sheared by passing the lysate through a 20-gauge needle attached to a sterile plastic syringe. Pass the lysate through the needle, at least 5 to 10 times, or until a homogenous lysate is achieved.

Protocol for the Isolation of RNA from Tissue

1. Depending on the disruption and homogenization method, add one of the following to a nuclease-free 1.5 ml microcentrifuge tube:
   – add 400 µl Lysis / Binding Buffer and the appropriate amount of frozen tissue (max. 20 – 25 mg)
   – disrupt and homogenize the tissue using a rotor-stator homogenizer (e.g., Ultra Turrax).

   i *Increasing the volume of Lysis buffer may be required to facilitate handling and minimize loss.*

   alternatively:
   – add 400 µl Lysis / Binding Buffer and the appropriate amount of tissue-powder (grinded with a mortar and pestle)
   – pass this lysate 5 – 10 times through a 20-gauge needle fitted to a syringe.

   i *For optimal yield do not exceed 10 mg tissue.*

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2. Centrifuge lysate for 2 minutes at maximum speed in a microcentrifuge and use only the collected supernatant for subsequent steps.

   i *Sometimes very small amounts of insoluble material will be present making this pellet invisible.*

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3. Add 200 µl (0.5 volumes) absolute ethanol to the lysate supernatant and mix well.

   i *If some lysate is lost during homogenization, reduce volume of ethanol accordingly.*

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4. Combine the High Pure Filter Tube with the Collection Tube and pipet the entire sample in the upper reservoir (maximal volume 700 µl).

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5. Centrifuge 30 seconds at maximal speed (13,000 × *g*) in a standard table top microcentrifuge.

   ⚠ ***After this centrifugation step, the glass fleece must be dry; if it looks wet, the centrifugation time must be increased.***

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6. – Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid.
   – Combine the Filter Tube with the used Collection Tube.

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7. – For each isolation pipet 90 µl DNase Incubation Buffer (white cap) into a sterile 1.5 ml reaction tube, add 10 µl DNase I working solution, mix.
   – Pipet the solution to the upper reservoir of the Filter Tube.
   – Incubate for 15 minutes at +15 to +25°C.

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8 – Add 500 µl Wash Buffer I (black cap) to the upper reservoir of the Filter Tube.
– Centrifuge 15 seconds at 8,000 × *g*.
– Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid.
– Combine the Filter Tube with the used Collection Tube.

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9 – Add 500 µl Wash Buffer II (blue cap) to the upper reservoir of the Filter Tube.
– Centrifuge 15 seconds at 8,000 × g and discard the flowthrough.
– Combine the Filter Tube with the used Collection Tube.

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10 – Add 300 µl Wash Buffer II (blue cap) to the upper reservoir of the Filter Tube.
– Centrifuge 2 minutes at full speed (approx 13,000 × *g*).

The extra centrifugation time ensures removal of residual Wash Buffer.

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11 – Carefully remove the column from the collection tube so that the column does not contact the flowthrough as this will result in carryover of ethanol.

Residual ethanol may interfere with subsequent reactions.
– Insert the Filter Tube into a nuclease free, sterile 1.5 ml microcentrifuge tube.

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12 – Add 100 µl Elution Buffer to the upper reservoir of the Filter Tube.
– Centrifuge the tube assembly for 1 minute at 8,000 × *g*.

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13 – The microcentrifuge tube now contains the eluted RNA.

Either use 10 µl of the eluted RNA directly in RT-PCR or store the eluted RNA at −80°C for later analysis.

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3. Results

Purity

Purified RNA is free of DNA, nucleases and all cellular and sample components that interfere with RT-PCR according to current Quality Control procedures. The absence of contaminating DNA is examined by a PCR without a preceding RT-reaction; no amplification product is obtained. The integrity and size distribution of total RNA purified with the High Pure RNA Tissue Kit have been checked by denaturing agarose gel electrophoresis and ethidium bromide staining. The relevant ribosomal species appear as sharp bands on the stained gel. 28 S ribosomal RNA band should be present at approximately twice the amount of the 18 S rRNA (mouse tissue).

Expected Yield

The concentration and purity of RNA can be determined by measuring the absorbance at 260 nm and 280 nm in a spectrophotometer. An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml. The ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity and should be 2.0 (RNA diluted in 20 mM Tris-HCl, pH 7.5).
The yield of total RNA depends on the starting material and varies depending on the amount of tissue used and the kind of disruption method applied. The yield with mouse muscle tissue could not be determined by spectrophotometer, but isolated RNA resulted in a specific RT-PCR signal.

Type of mouse tissue	Yield [µg / mg]
liver	0.5 – 2.8
kidney	0.5 – 1.0
spleen	0.5 – 3.0
lung	0.3 – 0.5
heart	0.3
muscle	n.d.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity	Kit stored under non-optimal conditions.	Store kit at +15 to +25°C at all times upon arrival.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	Store all buffers at +15 to +25°C.
		Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.
		After any lyophilized reagent is constituted, aliquot it and store the aliquot at −15 to −25°C.
	Ethanol not added to Wash Buffer I and/or II	Add absolute ethanol to the buffers before using.
		After adding ethanol, mix the buffers well and store at +15 to +25°C.
		Always mark Wash Buffer vials to indicate whether ethanol has been added or not.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
	Tissue stored and handled in less than optimal conditions	Use fresh tissue and disrupt immediately or flash frozen tissue stored at −60°C or below. Frozen tissue should not be allowed to thaw during handling prior to disruption in Lysis / Binding Buffer.
	Ethanol not added to the lysate in step 3	Addition of 0.5 volume of absolute ethanol to the lysate is necessary to promote selective binding of RNA to the glass fibers.
	High levels of RNase activity	Be careful to create an RNase-free working environment.
		Process starting material immediately or store it at −80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store it immediately at −80°C.
Tissue homogenate is viscous and difficult to pipet, low RNA yield	Insufficient disruption or homogenization	Add 350 µl of Lysis/Binding Buffer and repeat homogenization step to reduce viscosity.
	Too much starting material	Reduce amount of starting material and/or increase the amount of Lysis / Binding Buffer.
Clogged filter tube	Insufficient disruption and / or homogenization	Increase the disruption time for the rotorstator homogenizer or pass through syringe / needle additional times.
	Too much starting material	Reduce amount of starting material and/or increase the amount of Lysis / Binding Buffer.
DNA contamination	Lysis / Binding Buffer not completely removed from the glass fleece	Increase centrifugation time in step 5.
Absorbance ($A_{260\ nm}$) reading of product too high	Glass fibers, which might coelute with nucleic acid, scatter light	① Remove High Pure Filter Tube from tube containing eluted sample and spin sample for 1 minute at maximum speed. ② Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Samples “pop” out of wells in agarose gels	Eluate contains ethanol (from the Wash Buffer)	① After the wash step, do not let the flowthrough touch the bottom of the High Pure Filter Tube. ② Empty collection tube, reinsert filter tube in emptied collection tube, and recentrifuge for 30 seconds.

5. Additional Information on this Product

5.1. Test Principle

Isolating intact RNA is a prerequisite for the analysis of gene expression. Frequently applied techniques like reverse transcriptase-PCR (RT-PCR), northern blotting, and RNase protection require the use of intact undegraded RNA. Tissue samples are disrupted and homogenized in the presence of a strong denaturing buffer containing guanidine hydrochloride to instantaneously inactivate RNases, and to ensure isolation of intact RNA. After adding ethanol RNA binds selectively to a glass fiber fleece in the presence of a chaotropic salt (guanidine HCl). Residual contaminating DNA is digested by DNase I, applied directly on the glass fiber fleece. During a series of rapid "wash-and-spin" steps to remove contaminating cellular components the RNA remains bound to the glass fiber fleece. Finally, low salt elution removes the nucleic acids from the glass fiber. The process does not require RNA precipitation, organic solvent extractions, or extensive handling of the RNA.

① Samples (max. 25 mg tissue) are disrupted in Lysis / Binding Buffer and homogenized.

② RNA is isolated by binding to the glass fibers pre-packed in the High Pure Filter Tube.

③ Residual contaminating DNA is digested with DNase I.

④ Bound RNA is washed, thereby purified from salts, proteins and other cellular impurities.

⑤ Elution of the purified RNA with Elution Buffer.

5.2. Quality Control

- Tissue is disrupted and homogenized in Lysis/binding buffer and purified as described.
- RNA yield is determined by measuring the optical density at 260 nm.
- Integrity and size distribution are examined by the banding pattern of ribosomal RNA in a denaturing agarose gel.
- 10 µl of the RNA eluate is used in first strand synthesis with reverse transcriptase M-MuLV and $p(dT)_{15}$ as primer. In the following PCR, accomplished with Expand High Fidelity PCR System and specific primers for glycerinaldehyde-3-phosphate dehydrogenase (GAPDH), the expected amplification product of 983 bp is obtained.
- Absence of contaminating DNA is examined by a PCR without preceding RT reaction; no amplification product is obtained.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Trademarks

EXPAND and HIGH PURE are trademarks of Roche.
All third party product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to: **List of LifeScience products**

6.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

To call, write, fax, or email us, visit **sigma-aldrich.com** and select your home country to display country-specific contact information.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany