

# USER MANUAL

## InviTrap<sup>®</sup> RNA Cell HTS 96 Kit/ C

for purification of total RNA from up to max.  $5 \times 10^5$  human and animal cells  
in a 96-well format using a centrifuge without DNase digestion

## Instruction for InviTrap® RNA Cell HTS 96 Kit/ C

The **InviTrap® RNA Cell HTS 96 Kit/ C** is designed for isolation of high quality total RNA from 96 samples of human or animal cells. No enzymatic step (e.g. DNase-digestion) to remove contaminating genomic DNA is necessary. Due to the high purity, the isolated total RNA is ready to use for a broad panel of downstream applications.

The **InviTrap® RNA Cell HTS 96 Kit/ C** is optimized for use on a centrifuge. The kit is neither validated for the isolation of total RNA from serum, plasma, blood, tissue, nor for bacteria, yeast, or viruses. The performance of the kit in isolating and purifying total RNA from fecal samples has not been evaluated.



Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for *in vitro* diagnostic use in countries where the EU Directive 98/79/EC on *in vitro* medical devices is not recognized

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The Invisorb® technology is covered by patents and patent applications: US 6,110,363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviTrap® and Invisorb® are registered trademarks of Invitex Molecular GmbH.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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## Kit Content of InviTrap® RNA Cell HTS 96 Kit/ C

	24 x 96 RNA preps
Catalogue No.	7061300400
Lysis Solution S	3 x 250 ml
Binding Buffer R	2 x 105 ml (final volume 2 x 350 ml)
Wash Buffer R1	2 x 400 ml (final volume 2 x 800 ml)
Wash Buffer R2	3 x 200 ml (final volume 3 x 1000 ml)
Elution Buffer R	200 ml
DNA Binding Plate E	6 x 4
RNA Binding Plate D	6 x 4
2.0 ml Collection Plate	6 x 4
Elution Plate L	48
Plate-Lid	24
Initial steps	<p>Add 245 ml 96-100% Ethanol to each bottle <b>Binding Buffer R.</b></p> <p>Add 400 ml 96-100% Ethanol to each bottle <b>Wash Buffer R1.</b> Mix shortly and keep the bottle always firmly closed!</p> <p>Add 800 ml 96-100% Ethanol to each bottle <b>Wash Buffer R2.</b> Mix shortly and keep the bottle always firmly closed!</p>
<p><b>Adjust Lysis Solution S with 1/100 volume of 1M DTT.</b> Due to the instability of dissolved DTT under oxidative conditions, do not mix the whole <b>Lysis Solution S</b> with DTT. We recommend the preparation of a volume DTT-containing <b>Lysis Solution S</b> shortly before carrying out the purifications adapted to the number of samples that will be processed. Store the remaining <b>Lysis Solution S</b> and DTT separately in accordance to the storage instructions.</p> <p>It is possible to replace DTT by <math>\beta</math>-Mercaptoethanol. In that case, adjust <b>Lysis Solution S</b> with 1/100 volume of <math>\beta</math>-Mercaptoethanol as described above.</p>	

## Symbols

	Manufacturer
	Lot number
	Catalogue number
	Expiry date
	Consult operating instructions
	Temperature limitation
	Do not reuse
	Humidity limitation

**Attention:** Do not combine components of different kits, unless the lot numbers are identical!

## Storage

All buffers and kit contents of the InviTrap® RNA Cell HTS 96 Kit/ C should be stored at room temperature and are stable for at least 12 months.

**Room temperature (RT) is defined as range from 15-30°C.**

Before every use, make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by warming carefully (up to 30°C).

**Wash Buffers** charged with ethanol should be appropriately sealed and stored at room temperature.

**Binding Buffer** charged with ethanol should be appropriately sealed and stored at room temperature.

**Store 1 M DTT at - 20°C (not provided)**

## Quality control and product warranty

Invitek Molecular warrants the correct function of the **InviTrap® RNA Cell HTS 96 Kit/ C** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, Invitek Molecular will check the lot and if Invitek Molecular investigates a problem in the lot, Invitek Molecular will replace the Product free of charge.

Invitek Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all components of the **InviTrap® RNA Cell HTS 96 Kit/ C** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviTrap® RNA Cell HTS 96 Kit/ C** or other Invitek Molecular products, please do not hesitate to contact us. A copy of Invitek Molecular's terms and conditions can be obtained upon request or are presented at the Invitek Molecular webpage [www.invitek-molecular.com](http://www.invitek-molecular.com).

**For technical support or further information please contact:**

**from Germany** +49-(0)30-9489-2901/ 2910

**from abroad** +49-(0)30-9489-2907

**or contact your local distributor .**

## Intended use

The **InviTrap® RNA Cell HTS 96 Kit** is an optimized system for reliable and fast isolation of high quality total RNA from fresh or frozen human or animal cells (cell culture). For reproducible and high yields an appropriate sample storage and quick operation under the rules for RNA operation is essential (s. page 26). The purified RNA can be used for *in vitro* diagnostic analysis.

The **InviTrap® RNA Cell HTS 96 Kit/ C** is optimized for use with a centrifuge.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

*The kit complies with EU Directive 98/79/EC on in vitro medical devices. However, it is not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.*

## Product use limitation

The kit is neither validated for the isolation of total RNA from serum, plasma, blood, tissue, nor for bacteria, yeast, or viruses. The performance of the kit in isolating and purifying total RNA from fecal samples has not been evaluated.

The kit was not tested on its ability to desalinate RNA or for RNA purification from enzymatic reactions, like DNase digestion mixtures, Proteinase digestion, RNA ligation, or labeling reactions.

The included chemicals are for one time use, plates can be used several times, if not all wells were used in first run and unused wells were sealed during the process and no contamination occurred.

When changing the starting material or the flow trace, no guarantee in operability is issued.

The user is responsible to validate the performance of the Invitex Molecular kits for any particular use, since the performance characteristics of our kits have not been validated for all specific applications.

Invitex Molecular products may be used e.g. in clinical diagnostic laboratory systems under following conditions:

- If used in the US, based on the condition that the complete diagnostic system of the laboratory has been validated pursuant to CLIA' 88 regulations.
- For other countries based on the condition that the laboratory has been validated pursuant to equivalents according to the respective legal basis.

All products sold by Invitex Molecular are subjected to extensive quality control procedures (according to EN ISO 13485) and are warranted to perform as described when used correctly. Any problems should be reported immediately.

The chemicals and plastic parts are for laboratory use only, they must be stored in the laboratory and must not be used for purposes other than intended.

The kit contents are unfit for consumption.

## Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at [www.invitek-molecular.com](http://www.invitek-molecular.com) for each Invitek Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

Invitek Molecular has not tested the liquid waste generated by the **InviTrap® RNA Cell HTS 96 Kit** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **InviTrap® RNA Cell HTS 96 Kit** to which they apply are listed below as follows:

### Lysis Solution S



Warning

H315-H319-P280- P305+P351+P338

H302: Harmful if swallowed

H315: Causes skin irritation.

H319: Causes serious eye irritation

H412: Harmful to aquatic life with long lasting effects

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

EUH032: Contact with acids liberates very toxic gas.

### Wash Buffer R1



Warning

H302-H332-H412 P280- P305+P351+P338-EUH032

**Emergency medical information can be obtained 24 hours a day from infotrac:**

**outside of USA: 1 – 352 – 323 – 3500**

**in USA : 1 – 800 – 535 – 5053**

## Product characteristic of the InviTrap® RNA Cell HTS 96-Kit

Starting material	Yield	Time
up to 5 x 10 <sup>5</sup> human or animal cells (cultured cells)	max: 15-20 µg e.g. 10 - 15 µg total RNA from 5 x10 <sup>5</sup> NIH 3T3 fibroblasts 5 µg total RNA from 1 x 10 <sup>5</sup> MRC 5 cells	ca. 35 min using a centrifuge

The **InviTrap® RNA Cell HTS 96 Kit/ C** provides a fast and efficient way for the isolation of high quality total RNA from 96 – 192 RNA samples from max. 5 x 10<sup>5</sup> human or animal cells (cell culture).

The **InviTrap® RNA Cell HTS 96 Kit/ C** is designed for use on a centrifuge. In less than 35 min 192 samples can be processed gaining highly purified RNA.

Special buffer conditions guarantee an efficient lysis of the starting material and an inactivation of endogenous RNases. Genomic DNA contaminations are almost completely separated from the total RNA by binding to a carrier substance.

No enzymatic step, like DNase digestion, to remove contaminating genomic DNA is necessary. Subsequent RNA is bound onto the membrane of the RNA Binding Plate C. Contaminants are removed by following wash steps and high purified RNA can be eluted in small volume of RNase free water.

The extracted RNA contains enriched mRNA.

The procedures require minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedures are optimized to avoid sample-to-sample cross-contamination.

Due to the high purity, the isolated total RNA is ready to use for a broad panel of downstream applications (see below) or can be stored at  $-80^{\circ}\text{C}$  for subsequent use.

### Downstream Application

- RT-PCR
- DDRT-PCR
- Real-time PCR (quantitative RT-PCR, like TaqMan® und LightCycler® technologies)
- cDNA syntheses
- Mircoarray analysis
- Northern Blot analysis

### Principle and procedure

The InviTrap® RNA Cell HTS 96 Kit/ C procedure comprises following steps:

1. lysis of cells
2. selective binding of the genomic DNA to specific carrier and separation of contaminating DNA on the surface of the DNA Binding Plate E
3. remaining sample transferring into the wells of the RNA Binding Plate D, followed by the adjustment of the binding conditions
4. binding of the total RNA to the membrane
5. washing of the membrane and elimination of contaminants and ethanol
6. elution of high pure total RNA

After lysis, the DNA binds onto membrane of the DNA Binding Plate E, after contaminations and enzyme inhibitors are efficiently removed during the following two wash steps and highly purified RNA is eluted in Elution Buffer or water.

### Sampling and storage of starting material:

Best results are obtained using freshly extracted cells. As long as samples are not shock frosted or are inserted in RNase inhibitors or denaturing reagents, RNA is not secured. Therefore, it is essential, that cells are immediately flash frozen after cell harvest and are stored at  $-80^{\circ}\text{C}$ . RNA purification should be processed as soon as possible. Cells also can be stored in **Lysis Solution S** at  $-80^{\circ}\text{C}$  after cell lysis.

RNA from deep frozen samples is stable for months.

#### Lysis

After removing of the culture medium, cells are directly charged with **DTT**-containing **Lysis Solution S** and thoroughly mixed. Due to the strong denaturing conditions cells are quickly lysed, simultaneously RNases are inactivated. **DTT** is added to inactivate the RNases by cleaving intramolecular disulfide bridges.

#### Binding and removal of DNA

During lysis the DNA is bound to the carrier contained in **Lysis Solution S**.

Lysate will be completely transferred to a **DNA Binding Plate E** and centrifuged. DNA remains bound to the surface of the membrane of the **DNA Binding Plate E**. The RNA-containing solution is located in the eluate.

## Binding total RNA

After adding **Binding Buffer R** to the filtrates, those are completely transferred onto the RNA Binding Plate D and the RNA is bound on the membrane.

## Removing residual contaminants

Contaminants are efficiently washed away using **Wash Buffer R1** and **R2**, while the RNA remains bound to the membrane.

## Elution

Total RNA is eluted from the membrane using 60 - 80 µl Elution Buffer R (RNase free water). The eluted RNA is ready for use in different subsequent tests.

## Important notes

### Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify Invitex Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitex Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 5). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contaminations, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

## Sample quantity

The InviTrap® RNA purification procedure is optimized for the use of 50 to max.  $5 \times 10^5$  cells. The cell amount should not exceed, therefore direct cell counting is recommended. Table 1 may be used as a guide.

### Growth area and number of HeLa cells in various cell-culture plates

Cell culture plate	Growth area in cm <sup>2</sup> *	Number of cell**
96 well	0.3 – 0.6	$4-5 \times 10^4$
48 well	1.0	$1.3 \times 10^5$
12 well	4.0	$5.0 \times 10^5$
6 well	9.5	$1.2 \times 10^6$

\*) Growth area varies slightly depending on the supplier

\*\*) Confluent growth is assumed. Values are reported per well

The well membranes have a maximum binding capacity of approx. 100 µg, but actual yields will be different depending on the cell line even for the same amount of cells.

If more than  $5 \times 10^5$  cells are to be processed, it is recommend splitting the sample. Doubling the used buffer volumes prior RNA binding onto the membrane, does not lead to designated result in either case, particularly with respect to removal of contaminating DNA. Volumes of Wash- and Elution Buffer do not need to be aligned.

Depending on used cell line, lysates may become viscous using large cell quantity and this may cause clogged membranes. High viscosity always leads to lower yield and quality of isolated RNA. Therefore, it is recommended to perform preliminary viscosity analysis of the desired cell line depending on cell quantity, as well as analyzing RNA yield and purity. More than  $1 \times 10^6$  cells should not be used.

#### Average total RNA yield depending on cell line using the InviTrap® RNA Cell HTS 96 Kit

Cell Line	Source	yield in µg for $1 \times 10^5$ cells
HeLa	human cervical carcinoma	2.0
Jurkat	human T-cell leukemia	1.7
MRC 5	human	5.0
NIH3T3	human	6.5

#### Preparing reagents and buffers

24 x 96 total RNA-extractions:
add 245 ml 96-100% ethanol to the bottle <b>Binding Buffer R</b>
add 350 ml of 96-100% ethanol to each bottle <b>Wash Buffer R1</b> ; mix shortly and keep the bottle always firmly closed
add 800 ml of 96-100% ethanol to each bottle <b>Wash Buffer R2</b> ; mix shortly and keep the bottle always firmly closed
adjust <b>Lysis Solution S</b> with 1/100 volume of 1 M DTT*

#### Harvesting cells

**Cells grown in a monolayer:** Cells grown in a monolayer in 96-well plates can be lysed directly in the wells. Completely remove medium by pipetting

**Cells grown in suspension:** Transfer aliquots of up to  $5 \times 10^5$  cells into a 96-well plate. Centrifuge at  $240 \times g$  (1.500 rpm) for 5 min and completely remove medium by pipetting.

**Note:** *Incomplete removal of cell culture medium will inhibit partially lysis and dilute the lysate, which will affect the yield and quality of the resulting RNA.*

## Lysis Solution S

Shake **Lysis Solution S** gently before use to homogenize the carrier! Wait a short time because of foam formation!

Cell lysates stored in Lysis Solution S are stable at  $-80^{\circ}\text{C}$  for several months.

Adjust **Lysis Solution S** with 1/100 volume of 1 M **DTT**. Due to the instability of dissolved **DTT** under oxidative conditions, do not mix the whole **Lysis Solution S** with **DTT** in case of the kits with 50 and 250 preparations. We recommend the preparation of a volume **DTT-containing Lysis Solution S** shortly before carrying out the purifications adapted to the number of samples that will be processed. Store the remaining **Lysis Solution S** and **DTT** separately in accordance to the storage instructions (see "Storage", page 4).

It is possible to replace **DTT** by  $\beta$ -Mercaptoethanol. In that case, adjust **Lysis Solution S** with 1/100 volume of  $\beta$ -Mercaptoethanol as described above.

## Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). (See our web page: [www.invitex-molecular.com](http://www.invitex-molecular.com))

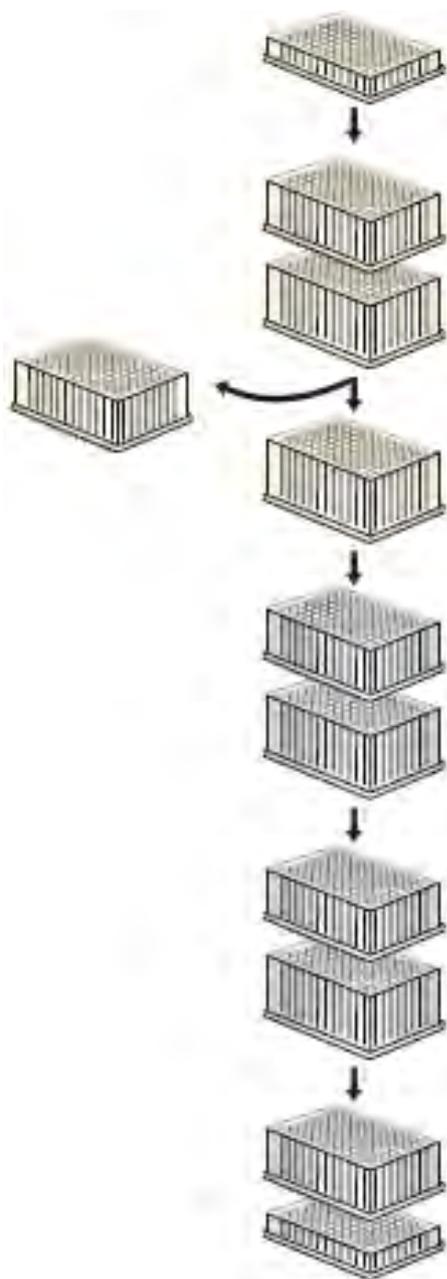
- Measuring cylinder (250 ml)
- Disposable gloves
- Multichannel pipet with tips
- Reagents reservoirs for multichannel pipets
- 96 - 100% ethanol
- optional: DNase A (10 mg/ml)
- Centrifuge: output  $\geq 2.000 \times g$  is necessary, for example Eppendorf Centrifuge 5804 / 5804 R / 5810 / 5810 R with Deepwell-Plate-Rotor (A-2-DWP)
- DTT

### Possible supplier for centrifuges

Eppendorf AG  
22331 Hamburg, Germany  
Phone: +49 (0) 40 53801 0  
Fax: +49 (0) 40 53801 556  
E-Mail: [eppendorf@eppendorf.com](mailto:eppendorf@eppendorf.com)  
Internet: [www.eppendorf.com](http://www.eppendorf.com)

SIGMA Laborzentrifugen GmbH  
37507 Osterode am Harz, Germany  
Phone: +49-5522-5007-0  
Fax: +49-5522-5007-12  
E-Mail: [info@sigma-zentrifugen.de](mailto:info@sigma-zentrifugen.de)  
Internet: [www.sigma-zentrifugen.de](http://www.sigma-zentrifugen.de)

## Scheme of the InviTrap® RNA Cell HTS 96 Kit/ C



### Please read protocols prior the start of the preparation

Removing the culture medium and cell extraction  
Add 250  $\mu$ l DTT-containing Lysis Solution S  
Mix thoroughly, 20 sec. up to 5 min.

Place the DNA Binding Plate E on an Elution Plate L  
Transfer lysates in DNA Binding Plate E  
Incubate for 1 min., seal with a Plate-Lid  
Centrifuge DNA Binding Plate E/ Elution Plate L for 3 min at 4.000 U/min

Discard DNA Binding Plate E

Add 230  $\mu$ l Binding Buffer R to the eluated lysate  
Mix thoroughly by pipetting up and down

Place RNA Binding Plate D on a 2.0 ml Collection Plate  
Transfer the lysates in the RNA Binding Plate D  
Incubate for 1 min., seal with the Plate-Lid  
Centrifuge RNA Binding Plate D/ 2ml Collection Plate for 3 min at 4.000 U/min

Add 500  $\mu$ l Wash Buffer R1, seal with the Plate-Lid  
Centrifuge RNA Binding Plate D/ 2ml Collection Plate for 3 min at 4.000 U/min  
remove Plate-Lid, discard filtrates

Add 600  $\mu$ l Wash Buffer R2, seal with the Plate-Lid  
Centrifuge RNA Binding Plate D/2ml Collection Plate for 3 min at 4.000 U/min

Add 600  $\mu$ l Wash Buffer R2, seal with Plate-Lid  
Centrifuge RNA Binding Plate D/2ml Collection Plate at min 4000 U/min for 3 min, discard filtrate, following centrifuge 10 min for ethanol removal, remove the Plate-Lid.

Place RNA Binding Plate D onto RNase free Elution Plate L  
Add 60-80  $\mu$ l Elution Buffer R, incubate for 2 min, seal with a Plate-Lid  
Centrifuge RNA Binding Plate D/ Elution Plate L for 3 min at 4.000 U/min  
Discard RNA Binding Plate D  
Seal Elution Plate L, place on ice or store at  $-80^{\circ}\text{C}$

## **Protocol: RNA Isolation from 50 – 1 x 10<sup>5</sup> human or animal cells using a centrifuge**

Please read the instructions carefully and conduct the prepared procedure.

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**Note:** *Shake Lysis Solution S gently before use! Wait a short time because of foam formation!  
Cell lysates in Lysis Solution S can be stored at –80°C for several months.*

1. Add 250 µl DTT-containing **Lysis Solution S** to each well of the Microtiter Plate with the cells. Shake thoroughly for 20 sec. up to 5 min while keeping the plate flat on the bench. Alternatively, it is possible to mix the samples thoroughly by using a shaker for microtiter plates or by pipetting up and down.
2. Place the DNA Binding Plate E on top of an Elution Plate L.
3. Apply the lysates (230 µl) from step 1 into the wells of the DNA Binding Plate E and incubate for 1 min.
4. Seal the DNA Binding Plate E with the Plate-Lid.
5. Load the DNA Binding Plate E/ Elution Plate L into the holder and place the whole assembly in the rotor bucket.
6. Centrifuge at 1.700 x g (4.000 rpm) for 3 min at room temperature (RT).
7. Discard the DNA Binding Plate E and take the Elution Plate L out of the centrifuge.

**Note:** *Take care not to wet the rims of the wells to avoid cross-contamination in this and in following steps.*

8. Add 230 µl of **Binding Buffer R** to the RNA-containing lysates into each well of the Elution Plate L and mix thoroughly by pipetting up and down.
9. Place the RNA Binding Plate D on top of a 2.0 ml Collection Plate.
10. Apply the lysates (460 µl) from step 8 into the wells of the RNA Binding Plate D. Seal the RNA Binding Plate D with the Plate-Lid and incubate for 1 min at RT.
11. Load the RNA Binding Plate D/ 2.0 ml Collection Plate into the holder and place the whole assembly in the rotor bucket. Centrifuge at 1.700 x g (4.000 rpm) for 3 min at RT. Take the RNA Binding Plate D/ 2.0 ml Collection Plate out of the centrifuge and remove the Plate-Lid.
12. Add 500 µl **Wash Buffer R1** to each used well of the RNA Binding Plate D. Seal the RNA Binding Plate D with the Plate-Lid. Load the RNA Binding Plate D/2.0 ml Collection Plate into the holder and place the whole assembly in the rotor bucket. Centrifuge at 1.700 x g (4.000 rpm) for 3 min at RT.
13. Take the RNA Binding Plate D out of the centrifuge, remove the Plate-Lid and place on a clean paper towel. Take the 2.0 ml Collection Plate out of the centrifuge and discard flow-through. Dry the upper site of the 2.0 ml Collection Plate with a clean paper towel.
14. Place the RNA Binding Plate D again on top of the 2.0 ml collection plate.

15. Add 600  $\mu$ l **Wash Buffer R2** to each used well of the RNA Binding Plate D. Seal the RNA Binding Plate D with the Plate-Lid. Load the RNA Binding Plate D/ 2.0 ml Collection Plate into the holder and place the whole assembly in the rotor bucket. Centrifuge at 1.700 x g (4.000 rpm) for 3 min at RT. Take the RNA Binding Plate D/ 2.0 ml collection plate out of the centrifuge and remove Plate-Lid.
16. Add again 600  $\mu$ l **Wash Buffer R2** to each used well of the RNA Binding Plate D. Seal the RNA Binding Plate D with the Plate-Lid. Load the RNA Binding Plate D/ 2.0 ml Collection Plate into the holder and place the whole assembly in the rotor bucket. Centrifuge for 3 min at 1.700 x g (4.000 rpm). Remove the filtrate and centrifuge for 10 min at RT at maximum speed (minimum 1.700 x g (4.000 rpm)) to eliminate any traces of ethanol.
17. Take the RNA Binding Plate D out of the centrifuge, place it on a clean paper towel and remove the Plate-Lid.
18. Place the RNA Binding Plate D on top of an RNase-free Elution Plate L. To elute, pipet 60-80  $\mu$ l **Elution Buffer R** (RNase-free) directly onto the membrane into each used well of the RNA Binding Plate D. Incubate for 2 min and seal the RNA Binding Plate D with the Plate-Lid.
19. Load the RNA Binding Plate D/ Elution Plate L into the holder and place the whole assembly in the rotor bucket. Centrifuge at 1.700 x g (4.000 rpm) for 3 min at RT. Discard the RNA Binding Plate D and take out the Elution Plate L of the centrifuge. Cover the Elution Plate L for storage. Store the extracted total RNA at  $-80^{\circ}\text{C}$ .

## Notes on usage of centrifuges

Using **InviTrap<sup>®</sup> RNA Cell HTS 96 Kit/ C** in the centrifuge RNA can be isolated in parallel 2 x 96 samples and is ready to use for subsequent downstream applications.

For optimal use of the kit and for realizing of reproducible high yields centrifuges should be used with 96-well deep well rotors, like the Sigma-Centrifuge 4-15C or Centrifuge 4K15C or the Eppendorf 5804 R/ 5810 R centrifuge with Deepwell-Plate-Rotor (A-2-DWP). Furthermore, an output above 1.700 x g (4.000 rpm) is necessary.

**Important:** *All centrifugation steps are performed at RT.  
Under no circumstances use plate rotor without 96 well plates, or with plates with varying buffer amounts never use only 1 plate in the centrifuge. Due to the unbalance the rotor would be destroyed under the high centrifugal forces.*

## Troubleshooting

Problem	Cause	Comments and suggestions
<b>little or no total RNA eluted</b>	insufficient disruption or homogenisation	reduce amount of starting material. Overloading reduces yield
	incomplete elution	prolong the incubation time with <b>Elution Buffer R</b> to 5-10 min or repeat elution step once again
	buffer temperature too low	all buffers must be at room temperature throughout the procedure
	unclean working, RNA digestion	use RNase free tubes and pipet tips (see Handling RNA in the Appendix)
	inappropriate sample extraction or storage	ensure that sample is flash frozen immediately after extraction; store at $-80^{\circ}\text{C}$
<b>clogged Plate Filter</b>	insufficient disruption or homogenization	after lysis spin lysate to pellet debris and continue with the protocol using the supernatant.
	too much starting material	reduce amount of starting material.
<b>degraded RNA</b>	incorrect storage of starting material	ensure that the starting material is frozen immediately in liquid $\text{N}_2$ and is stored continuously at $-80^{\circ}\text{C}$ avoid thawing of the material; ensure that the protocol, especially the first steps, has been performed quickly.
	RNase contaminations of solutions, receiver tubes etc.	use sterile, RNase-free filter-tips, before every preparation clean up the pipettes, the devices and the working place; always wear gloves
	<b>Lysis Solution S</b> does not contain <b>DTT</b>	ensure that <b>DTT</b> has been added to the <b>Lysis Solution S</b>
<b>DNA-contamination</b>	no optimal homogenization of DNA binding carrier	shake carefully <b>Lysis Solution</b> before use
	too much starting material	reduce amount of starting material. DNase digestion of the eluate containing the total RNA
	elution volume too low	use elution volumes of 60 – 80 $\mu\text{l}$ , repeat elution step
	incomplete removal of DNA binding carrier	follow exactly the protocol and ensure the removal of the DNA binding carrier
	no DNase treatment	if necessary after the InviTrap RNA <sup>®</sup> 96 procedure, DNase digestion of the eluate containing the total RNA. After inactivating DNase by heat treatment, the RNA can be either used directly in the subsequent application without further treatment, or repurified using the RNA cleanup protocol
<b>low <math>A_{260}/A_{280}</math> value</b>	improper pH	use 10 mM Tris-Cl, not RNase-free water, to dilute the sample before measuring the purity
<b>low well-to-well reproducibility</b>	elution volume too low	use elution volumes of 60 – 80 $\mu\text{l}$ , repeat elution step (see protocols)
	inhomogeneous cell growth	ensure, that cells grow equably

## Appendix

### General notes on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases, which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases has to be reduced as much as possible. Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification is to be carried out.

All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving alone will not completely inactivate many RNases. Oven baking will both inactivate RNases and ensure that no other nucleic acids (such as Plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware must stand 12 hours at 37°C and then be autoclaved or heated to 100°C for 15 min to remove residual DEPC.

- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
- Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plasticware rinsed with chloroform to inactivate RNases.
- All buffers must be prepared with RNase-free ddH<sub>2</sub>O.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Change gloves frequently and keep tubes closed.
- All centrifugation steps are carried out at room temperature.
- To avoid cross contamination cavity seams should not be covered with fluid.
- Reduce the preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase free).
- Keep isolated RNA on ice.
- Do not use kit components from other kits with the kit you are currently using, unless the lot numbers are identical.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by personnel trained in *in vitro* diagnostic laboratory practice

### Storage of RNA

Purified RNA can be stored at -80°C and is stable for several years at this condition.

### Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm ( $A_{260}$ ) in photo spectrometer. Readings should be greater than 0.15 to ensure significance. An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml. This relation is valid only for measurements at neutral pH. The ratio between absorbance values at 260 nm and 280 nm gives an estimate of RNA purity (see below).

When measuring RNA samples, make sure that cuvettes are RNase-free, esp. if the RNA is to be recovered after photo spectrometry. This can be accomplished by washing cuvettes with 0.1 NaOH, 1 mM EDTA followed by washing with RNase-free water. Use buffer in which the RNA is diluted to zero the photo spectrometer.

An example of the calculation involved in RNA quantification:

Volume of RNA sample 100  $\mu$ l

Dilution = 20  $\mu$ l of RNA sample + 180  $\mu$ l of 10 mM Tris, pH 7.0 (1/10 dilution).

Measure absorbance of diluted sample in a 0.2 ml cuvette (RNase free)

$A_{260} = 0.2$

Concentration of RNA sample = 40  $\mu$ g/ml \*  $A_{260}$  \* dilution factor  
= 40  $\mu$ g/ml \* 0.2 \* 10  
= 80  $\mu$ g

Total amount = concentration \* volume of sample in ml  
= 80  $\mu$ g/ml \* 0.1 ml  
= 8  $\mu$ g of RNA

## Purity of RNA

The ratio of the readings at 260 nm and 280 nm ( $A_{260}/A_{280}$ ) provides an estimate of the purity of RNA with respect to the contaminants that absorb in the UV, such as protein. However, the  $A_{260}/A_{280}$  ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting  $A_{260}/A_{280}$  ratio can vary greatly. Lower pH results in lower  $A_{260}/A_{280}$  ratio and reduced sensitivity to protein contamination.\* For accurate values, it is recommend to measure absorbance in 10 mM Tris Cl, pH 7.5. Pure RNA has an  $A_{260}/A_{280}$  ratio of 1.9-2.1\*\* in 10 mM Tris Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution.

For determination of RNA concentration, however, it is recommend diluting the sample in a buffer with neutral pH since the relationship between absorbance and concentration ( $A_{260}$  reading of 1 = 40  $\mu$ g/ml of RNA) is based on an extinction coefficient calculated for RNA at neutral pH.

\* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of Ph and ionic strength on the spectrophotometric assessment of nucleic acid purity. Bio Techniques 22, 474

\*\* Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris Cl, pH 7.5) with some photo spectrometers

## Ordering information

Product	Package size	Catalogue No.
InviTrap <sup>®</sup> RNA Cell HTS 96 Kit / C	24 x 96 preps	7061300400
InviTrap <sup>®</sup> Spin Cell RNA Mini Kit	250 preps	1061100300

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