RIDA® Xtract

Art. Nr.: PGZ001
250 Preparations

For *in vitro* diagnostic use.

15 – 30 °C

R-Biopharm AG, An der neuen Bergstraße 17, D-64297 Darmstadt, Germany
Tel.: +49 (0) 61 51 81 02-0 / Telefax: +49 (0) 61 51 81 02-20

1. Intended use

For *in vitro* diagnostic use. RIDA® Xtract is a spin-filter based extraction kit for the simultaneous isolation and purification of high quality bacterial and viral DNA as well as viral RNA from human serum, plasma specimen, cerebral fluid, cell culture supernatant, other cell free body fluids (e.g. urine), swabs, tissue biopsies and stool specimen.

2. Test principle

The RIDA® Xtract kit offers the advantage of the simultaneous isolation and purification of viral DNA/RNA and bacterial DNA from a diverse range of fresh and frozen sample materials. This enables to test the sample for all kinds of nucleic acids of viruses and bacteria after one preparation.

Using the RIDA® Xtract Kit a diverse range of sample materials are transferred into the Reaction Tubes together with a specially designed Dilution Buffer to adjust to a final sample volume of 400 µl. The prefilled buffer and enzymes lyse the samples, stabilize the nucleic acids and enhance the viral and bacterial DNA and/or RNA adsorption to the membrane of the Spin Filter. Contaminants are removed by repeated washing steps and the purified nucleic acids can be eluted in a small volume of Elution Buffer.

The RIDA® Xtract procedure comprises the following steps:

1. Lysis at different temperatures
2. Adjustment of the binding conditions
3. Binding of the nucleic acids in the lysate to the membrane of a Spin Filter
4. Washing of the membrane and elimination of contaminants and ethanol
5. Elution of the nucleic acids

Lysis and adjustment of the binding condition

Samples are lysed under anti-chaotropic conditions at different, temperatures and under continuous shaking. Lysis is performed in the presence of Lysozym to break the cell wall of the bacteria, a Lysis Buffer and Proteinase K to digest the proteins. All is provided prefilled in the Reaction Tube. Unlysed sample parts should be removed before the binding step. Due to the strong denaturing lysis conditions RNases and DNases are inactivated simultaneously.
The addition of Carrier RNA (provided in the Reaction Tube) is necessary for the enhancement of viral DNA/RNA recovery so that very small numbers of viral

DNA/RNA molecules will also be purified. Carrier RNA also stabilizes nucleic acids in samples with very small nucleic acid concentrations.

Binding nucleic acids

After adding Binding Solution to adjust optimal binding conditions, the lysate will be applied onto the Spin Filter and the nucleic acids are bound to the surface of the Spin Filter membrane as the lysate is drawn through by centrifugation.

Removing residual contaminants

Contaminants are efficiently washed away using Wash Buffer 1 and Wash Buffer 2, while the nucleic acids remain bound to the membrane of the Spin Filter.

Elution

High quality viral DNA/RNA and genomic DNA is eluted from the membrane using Elution Buffer or RNase free water. Eluting twice with 100 µl each time leads to a small increase in DNA yield. The Usage of small elution volumes may raise the DNA/RNA concentration. Elution volumes should be at least 40 µl. The volume of eluate recovered may be up to 5 µl less than the volume of elution buffer applied to the Spin Filter. The volume of eluate recovered depends on the nature of the sample. The eluted DNA/RNA is ready to use in different subsequent applications.

This manual contains 5 protocols:

Protocol 1: Isolation of DNA and/or RNA from stool samples

Protocol 2: Simultaneous isolation of total nucleic acids (DNA and RNA) from cell free body fluids

Protocol 3: Simultaneous isolation of total nucleic acids (DNA and RNA) from swab material

Protocol 4: Simultaneous isolation of total nucleic acids (DNA and RNA) from tissue biopsies

Protocol 5: Isolation of DNA from bacterial pellets (1 x 10^9 bacteria cells)

3. Reagents provided

Tab.1: Reagents provided (Reagents provided in the kit are sufficient for 250 extractions)

Kit Component	Amount	Description
Reaction Tubes	5x 50	Reaction Tubes
Dilution Buffer	1x 150 ml	Dilution Buffer
Binding Solution	1x empty bottle (final volume 1 x 120 ml)	Binding Solution
Wash Buffer 1	2x 40 ml (final volume 2 x 80 ml)	Wash Buffer 1
Wash Buffer 2	2x 30 ml (final volume 2 x 150 ml)	Wash Buffer 2
Elution Buffer	1x 60 ml	Elution Buffer
Spin Filter	5x 50	Spin Filter
Collection Tubes	15x 50	Collection Tubes
Elution Tubes	5x 50	Elution Tubes

Note: *Store all kit components at room temperature (15 - 30 °C)!*

4. Storage instructions

- All buffers and kit components of the RIDA® Xtract kit including the Reaction Tube (incl. Lysis Buffer, Proteinase K, Lysozyme and Carrier RNA) should be stored well sealed and dry at room temperature (15 - 30 °C) and can be used until the expiration date. After expiry the quality is no longer guaranteed.
- If the kit is opened before 12 months remaining expiry, the expiry will be reduced to 12 months.
- Wash Buffer 1 and 2 filled with ethanol should be appropriately sealed.
- Binding Solution filled with Isopropanol should be appropriately sealed.
- Before every use make sure that all components have room temperature (15 - 30 °C). If there are any precipitates within the provided solutions, solve these precipitates by warming up carefully.

5. Additional equipment and materials required

- Microcentrifuge
- Heating block (37 - 95 °C)
- ddH_2O
- Ethanol (96 - 100 %)
- Isopropanol (molecular biological grade; e.g. Carl Roth, 2-Propanol Cat. no. 6752, Applichem Cat. No. A3928 or Sigma Cat. no. 59304-1L-F)
- 1.5 ml reaction tubes

- 2.0 ml reaction tubes (optional)
- Measuring cylinder (250 ml)
- Vortexer
- Pipettes (20 - 200 µl, 100 - 1000 µl)
- Filter tips
- Powder-free disposal gloves

6. Precautions for users

- For *in vitro* diagnostic use only.
- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- The RIDA® Xtract kit must only be handled by laboratory personnel trained in molecular biology methods.
- Strictly follow the working instructions.
- When and while handling with samples and RIDA® Xtract reagents, always wear a suitable lab coat, disposable gloves and protective goggles. After finishing the RIDA® Xtract procedure, wash your hands.
- Avoid skin contact.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- Samples must be treated as potentially infectious as well as all reagents, materials being exposed to the samples and liquid waste generated by the RIDA® Xtract kit and have to be handled according to the national safety regulations.
- The procedure requires minimal interaction by the user, allowing safe handling of potentially infectious samples.
- To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.
- RIDA® Xtract and its contents are unfit for consumption.
- The RIDA® Xtract reagents and the plastic parts are for laboratory use only. They must be stored in the laboratory and must not be used for purposes other than intended.
- If buffer bottles are damaged or leaking, wear disposable gloves and protective goggles when discarding the bottles in order to avoid any injuries.
- Do not use the kit after the expiration date.

7. Important Notes

7.1 Preparation of reagents

Binding Solution:
Fill 120 ml Isopropanol (molecular biologic grade) into the empty Binding Solution bottle (see chapter 5. Additional equipment and materials required).

Wash Buffer 1:
Add 40 ml of ethanol (96 - 100 %) to the Wash Buffer 1 bottles, mix thoroughly and always keep the bottles firmly closed.

Wash Buffer 2: Add 120 ml of ethanol (96 - 100 %) to the Wash Buffer 2 bottles, mix thoroughly and always keep the bottles firmly closed.

7.2 Handling of Spin Filters

Due to the sensitivity of DNA/RNA amplification technologies, the following precautions are necessary when handling Spin Filter to avoid cross-contamination between sample preparation.

- Carefully apply the sample or solution to the Spin Filter. Pipet the sample into the filter without wetting the rim of the column.
- Always change pipet tips between liquid transfers. We recommend the use of aerosol barrier pipet tips.
- Avoid touching the Spin Filter membrane with the pipet tip.

7.3 Sampling and storage of starting material

Stool Samples

Best results are obtained with fresh material, material that has been stored at 2 - 8 °C for up to 3 days or material that has been immediately frozen and stored at -20 or -80 °C.

Cultivated bacteria

Bacteria have to be pelleted after cultivation. Best results are obtained with fresh material or material that has been immediately frozen and stored at -20 °C or -80 °C. Repeated freezing and thawing of stored samples should be avoided since this leads to reduced DNA size.

Biopsy material/Tissue

Best results are obtained with fresh material or material that has been immediately frozen and stored at -20 °C or -80 °C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor quality starting material also leads to reduced DNA length and influences yield of purified DNA. The amount of purified DNA from tissue sample (max. 10 mg) depends on the nature of starting material.

Urine

The bacteria must be pelleted while the supernatant is completely removed (urea contaminations can inhibit PCR reactions). Best results are obtained with fresh peletted material or bacteria pellets that have been immediately frozen and stored at -20 °C or -80 °C. Repeated freezing and thawing of stored samples should be avoided since this leads to reduced DNA size. The amount of purified DNA from urine (max. 15 - 50 ml), depends on the included bacteria titre.

Swabs, Saliva

The protocol works with fresh saliva, prepared swabs as well as with dried swabs. The protocol has not been validated for isolation of DNA from swabs, which are stored under special storage buffers of other providers.
Best results are obtained using freshly extracted samples. As long as the samples are not snap frozen with liquid nitrogen or are incubated with RNase inhibitors or denaturing reagents, the viral RNA is not stable. Therefore it is essential, that samples are immediately snap frozen subsequent to the harvesting by using liquid nitrogen and are stored at -80 °C. Viral RNA in snap frozen samples is stable for months. Viral RNA purification should be processed as soon as possible. Samples can also be stored in the dissolved Lysis Buffer in the Reaction Tube for 1 h at room temperature, overnight at 4 °C, and for long term storage at -80 °C. Storage under deep frozen conditions is recommended.

Serum and Plasma (and other cell free body fluids)

Following centrifugation, plasma or serum from blood treated with anticoagulants like EDTA or citrate, *but not with heparin*, can be stored at 2 - 8 °C for up to 6 hours. For long-term storage, freezing at -20 – -80 °C in aliquots is recommended. Repeated freezing and thawing cycles must be avoided because denaturation and precipitation of proteins results in a decrease of the virus titer and thereby reduces the yield of the extracted viral RNA. Occurring cryoprecipitates can be pelleted by briefly centrifuging

(6,800 x g for 3 minutes). The cleared supernatant should be removed without disturbing the pellet and should be processed immediately. This step will not reduce viral titers.

Cell Culture Supernatants

Best results are obtained with fresh material or material that has been immediately frozen and stored at -20 °C or -80 °C after retrieving the cell culture supernatant. Repeated freezing and thawing of stored samples can influence the sensitivity.

7.4 Sample size of starting material

The RIDA® Xtract kit has been optimized for use of up to 200 µl of cell free body fluids, swab material, cell culture supernatants, up to 400 µl of rinse liquid from swabs, 1 x 10^9 mammalian cells, max. 10 mg tissue samples and 200 µl of stool supernatant.

7.5 Internal control (IC)/Extraction control

Internal Controls (IC) from the PCR assay provider can be used as extraction controls, if the fragments are longer than 100 bp.

Note: **Do not add these Internal Controls directly to the specimen!**

8. Protocols

The following notes are valid for all protocols:

- Ensure that Binding Solution, Wash Buffer 1 and Wash Buffer 2 have been prepared according to the instructions on the labels.
- Mix all buffers before use
- The DNA/RNA can also be eluted with a lower (but not lower than 40 µl) or a higher volume of Elution Buffer (In dependence of the expected yield or required concentration of the DNA/RNA).
- The eluate contains viral DNA and/or viral RNA as well as sometimes genomic DNA.
- After extraction, place the Elution Tube on ice. Store the nucleic acids for long term storage at -20 °C or -80 °C.
- The centrifugation steps were carried out with the Centrifuge 5415 D from Eppendorf. The indicated rpm amounts are referring to this centrifuge.

8.1 Protocol 1: Isolation of DNA and/or RNA from stool samples

Important Note:

- *Please read the protocols carefully prior to the start of the preparation procedure.*
- *Prewarm the needed amount of Elution Buffer to 80 °C for the final elution step.*

8.1.1 Sample Preparation

RNA: Dilute stool sample 1:10 with water. Vortex sample for 30 seconds. Centrifuge sample for 1 minute at 12,000 rpm.

DNA: Dilute stool sample 1:3 with water. Vortex sample for 30 seconds. Centrifuge sample for 30 seconds at 3,000 rpm.

DNA and RNA: Dilute stool sample 1:3 with water. Vortex sample for 30 seconds. Centrifuge sample for 30 seconds at 3,000 rpm.

8.1.2 Sample Lysis

- Transfer 200 µl of the sample supernatant into the provided Reaction Tube and add 200 µl Dilution Buffer.
  Optional: Add 20 µl RIDA®GENE ICR/ICD as extraction control.
- Close the cap and vortex shortly for 10 seconds and incubate for 10 minutes at RT.

8.1.3 Binding of the DNA and RNA

- Add 400 µl Binding Solution to the provided Reaction Tube and mix the sample completely by pipetting up and down or by vortexing.
- Transfer the sample into the Spin Filter. Close the cap, incubate for 1 minute and centrifuge for 1 minute at 9,300 x g (10,000 rpm).
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.

8.1.4 First Washing of the Spin Filter

- Add 500 µl Wash Buffer 1 to the Spin Filter and centrifuge at 9,300 x g (10,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.

8.1.5 Second Washing of the Spin Filter

- Add 700 µl Wash Buffer 2 to the Spin Filter and centrifuge at 9,300 x g (10,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.
- Remove the residual ethanol by final centrifugation for 4 minutes at maximum speed. Discard the Collection Tube with filtrate.

8.1.6 Elution of the DNA/RNA

- Place the Spin Filter into an Elution Tube.
- Add 60 µl of the Elution Buffer (prewarmed to 80 °C) directly onto the Spin Filter membrane.
- Incubate for 3 minutes at RT and centrifuge at 9,300 x g (10,000 rpm) for 1 minute.
- Discard the Spin Filter and place the Elution Tube with the eluted NA immediately on ice.

8.2 Protocol 2: Simultaneous isolation of total nucleic acids (DNA and RNA) from cell free body fluids

Important Note:

- *Please read the protocols carefully prior to the start of the preparation procedure.*
- *Prewarm the needed amount of Elution Buffer to 65 °C for the final elution step.*

8.2.1 Sample Preparation

The protocol has been optimized for the isolation of total nucleic acids from body fluids of 200 µl. For samples, which have a smaller volume than 200 µl, please fill up to a total volume of 400 µl with ddH_2O.

8.2.2 Sample Lysis

- Mix 200 µl of the sample with 200 µl of ddH_2O.
- Transfer the sample into the provided Reaction Tube. Close the cap and vortex shortly.
- Place the Reaction Tubes into a heating block and incubate under continuous

shaking for 15 minutes at 65 °C and for 10 minutes at 95 °C, which leads to higher sensitivity

8.2.3 Binding of the DNA/RNA

- Add 400 µl Binding Solution to the provided Reaction Tube and mix the sample completely by pipetting up and down or by vortexing.
- Transfer the sample into the Spin Filter. Close the cap, incubate for 1 minute and centrifuge for 2 minutes at 11,000 x g (11,000 rpm).
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.

8.2.4 First Washing of the Spin Filter

- Add 500 µl Wash Buffer 1 to the Spin Filter and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.

8.2.5 Second Washing of the Spin Filter

- Add 700 µl Wash Buffer 2 to the Spin Filter and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube. Remove the residual ethanol by final centrifugation for 4 minutes at maximum speed. Discard the Collection Tube with filtrate.

8.2.6 Elution of the DNA/RNA

- Place the Spin Filter into an Elution Tube.
- Pipet 60 µl of the Elution Buffer (prewarmed to 65 °C) directly onto the Spin Filter membrane.
- Incubate for 3 minutes at RT and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Spin Filter and place the Elution Tube with the eluted NA immediately on ice.

8.3 Protocol 3: **Simultaneous isolation of total nucleic acids (DNA and RNA) from swab material**

Important Note:

- *Please read the protocols carefully prior to the start of the preparation procedure.*
- *Prewarm the needed amount of Elution Buffer to 65 °C for the final elution step.*

8.3.1 Sample Preparation

8.3.1.1 For gram-positive bacteria

- Place the swab into the Reaction Tube, add 400 µl Dilution Buffer and mix by stirring with the swab. Break or cut the swab and close the tube.
- Incubate the sample in a heating block for 10 minutes at 37 °C followed by 65 °C for 10 minutes. (Continuous shaking increases the lysis procedure. It is possible to switch the heating block to 65 °C after 8 minutes while the sample is shaking. If the heating block heats faster than 4 °C per minute, the incubation time is 12 minutes. If the heating block has a slower heating rate, the incubation time has to be elongated).
- Remove the swab and continue with **chapter 8.3.2**.

8.3.1.2 For gram-negative bacteria

- Place the swab into the Reaction Tube and add 400 µl Dilution Buffer and mix by stirring with the swab. Break or cut the swab and close the tube.
- Incubate the sample in a heating block for 10 minutes at 65 °C (continuous shaking increases the lysis procedure).
  Remove the swab and continue with **chapter 8.3.2**.

8.3.1.3 For viral DNA and/or RNA

- Place the swab into the provided Reaction Tube and add 400 µl Dilution Buffer and mix by stirring with the swab. Break or cut the swab and close the tube.
- Place the Reaction Tube into a heating block and incubate for 15 minutes at 65 °C (continuous shaking increases the lysis procedure).
  Remove the swab and continue with **chapter 8.3.2**.

Note: *To get maximum yield of bacterial and viral nucleic acids it is essential to leave the swab in the reaction tube during the complete lysis time. It is possible to cut the swab shaft, so that you can close the cap of the Reaction Tube. Removing the swab from the Reaction Tube ahead of time will lead to a dramatically reduced final yield !*
After lysis time, carefully squeeze out the swab on the wall of the tube and discard the swab.

8.3.2 Sample Lysis

- Place the Reaction Tube into a heating block and incubate at 95 °C for 5 - 10 minutes (continuous shaking increases the lysis efficiency).

8.3.3 Binding of the DNA/RNA

- Add 400 µl Binding Solution to the sample and mix the sample completely by pipetting up and down or by vortexing.
- Transfer the sample into the Spin Filter. Close the cap and incubate for 1 minute. Centrifuge at 11,000 x g (11,000 rpm) for 2 minutes.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.

8.3.4 First Washing of the Spin Filter

- Add 500 µl Wash Buffer 1 to the Spin Filter and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate place the Spin Filter into a new Collection Tube.

8.3.5 Second Washing of the Spin Filter

- Add 700 µl Wash Buffer 2 to the Spin Filter and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube. Remove the residual ethanol by final centrifugation for 4 minutes at maximum speed.
- Discard the Collection Tube with filtrate.

8.3.6 Elution of the DNA/RNA

- Place the Spin Filter into a 1.5 ml Elution Tube
- Pipet 60 - 200 µl of Elution Buffer (prewarmed to 65 °C) directly onto the Spin Filter membrane.
- Incubate for 3 minutes at RT and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Spin Filter and place the Elution Tube with the eluted NA immediately on ice.

8.4 Protocol 4: Simultaneous isolation of total nucleic acids (DNA and RNA) from tissue biopsies

Important Note:

- *Please read the protocols carefully prior to the start of the preparation procedure.*
- *Prewarm the needed amount of Elution Buffer to 65 °C for the final elution step.*

8.4.1 Sample Preparation

- Transfer 1 - 10 mg of the tissue sample into the provided Reaction Tube.
- Add 400 µl of Dilution Buffer. Close the cap and vortex shortly.
- Place the Reaction Tube into a heating block and incubate under continuous shaking for 30 - 60 min at 56 °C.

8.4.2 Sample Lysis

- Place the Reaction Tube into a heating block and incubate for 5 - 10 min at 95 °C (continuous shaking increases the lysis efficiency).

***Note**:* Lysis times may be increased, if the lysis is not complete.

Important Note: *A longer lysis time could reduce the final yield and the quality of some viral RNA species*.

- After lysis, centrifuge the sample at max. speed for 1 minute to spin down unlysed material. Transfer the cleared supernatant completely into a 1.5 ml reaction tube (not provided).

8.4.3 Binding of the DNA/RNA

- Add 400 µl Binding Solution to the 1.5 ml reaction tube and mix the sample completely by pipetting up and down or by vortexing.
- Transfer the sample into the Spin Filter. Close the cap and centrifuge for 2 minutes at 11,000 x g (11,000 rpm).
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.

8.4.4 First Washing of the Spin Filter

- Add 500 µl Wash Buffer 1 to the Spin Filter and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.

8.4.5 Second Washing of the Spin Filter

- Add 700 µl Wash Buffer 2 to the Spin Filter and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube. Remove the residual ethanol by final centrifugation for 4 minutes at maximum speed.
- Discard the Collection Tube with filtrate.

8.4.6 Elution of the DNA/RNA

- Place the Spin Filter into an Elution Tube.
- Add 60 µl of the Elution Buffer (prewarmed to 65 °C) directly onto the Spin Filter membrane.
- Incubate for 3 minutes at RT and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Spin Filter and place the Elution Tube with the eluted NA immediately on ice.

8.5 Protocol 5: Isolation of DNA from bacterial pellets (1 x 10^9 bacteria cells)

Important Note:

- *Please read the protocols carefully prior to the start of the preparation procedure.*
- *Prewarm the needed amount of Elution Buffer to 65 °C for the final elution step.*

8.5.1 Sample Preparation

- Take an aliquot of the bacterial culture and spin it down at 11,000 x g (11,000 rpm) for 3 minutes.
- Remove the complete supernatant carefully.

8.5.1.1 For gram-positive bacteria

- Add 400 µl Dilution Buffer to the pellet and resuspend the pellet by pipetting up and down.
- Transfer the resuspended sample into the Reaction Tube and vortex shortly.
- Incubate the sample in a heating block for 10 minutes at 37 °C and at 65 °C for 10 minutes (Continuously shaking increases the lysis procedure. It is possible to switch the heating block to 65 °C after 8 minutes while the sample is shaking. If the heating block heats faster than 4 °C per minute, the incubation time is 12 minutes. If the heating block has a slower heating rate, the incubation time has to be elongated).
- Continue with **chapter 8.5.2**.

8.5.1.2 For gram-negative bacteria

- Add 400 µl Dilution Buffer to the pellet and resuspend the pellet by pipetting up and down.
- Transfer the resuspended sample into the Reaction Tube and vortex shortly. Incubate the sample in a heating block at 65 °C for 10 minutes (continuous shaking increases the lysis procedure).
- Continue with **chapter 8.5.2.**

8.5.2 Sample Lysis

- Place the Reaction Tube into a heating block and incubate at 95 °C for 5 - 10 minutes (continuous shaking increases the lysis efficiency).

8.5.3 Binding of the DNA

- Add 400 µl Binding Solution to the sample and mix the sample completely by pipetting up and down or by vortexing.
- Transfer the sample into the Spin Filter. Close the cap and centrifuge for 2 minutes at 11,000 x g (11,000 rpm).
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.

8.5.4 First Washing of the Spin Filter

- Add 500 µl Wash Buffer 1 to the Spin Filter and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube.

8.5.5 Second Washing of the Spin Filter

- Add 700 µl Wash Buffer 2 to the Spin Filter and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Collection Tube with filtrate and place the Spin Filter into a new Collection Tube. Remove the residual ethanol by final centrifugation for 4 minute at maximum speed.
- Discard the Collection Tube with filtrate.

8.5.6 Elution of the DNA/RNA

- Place the Spin Filter into an Elution Tube.
- Add 200 µl of the Elution Buffer (prewarmed to 65 °C) directly onto the Spin Filter membrane.
- Incubate for 3 minutes at RT and centrifuge at 11,000 x g (11,000 rpm) for 1 minute.
- Discard the Spin Filter and place the Elution Tube with the eluted NA immediately on ice.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (dependent on the expected yield of bacterial DNA).*

9. Troubleshooting

Problem	Probable cause	Comments and suggestions
clogged Spin Filter	insufficient lysis, homo-genization and/or too much starting material	increase lysis time increase g-force and / or centrifugation time reduce amount of starting material all centrifugation steps should be conducted at room temperatures
low amount of extracted DNA/RNA	insufficient lysis incomplete elution insufficient mixing of the sample with **Binding Solution** incomplete removal of cell culture medium	increase lysis time reduce amount of starting material; overloading of **Spin Filter** reduces yield prolong the incubation time with prewarmed **Elution Buffer** to 5 -10 min. perform the elution steps twice. take higher volume of **Elution Buffer** mix sample sufficiently by pipetting up and down with **Binding Solution** prior transfer of the sample onto the **Spin Filter** make sure that the cell culture medium is complete removed after the cell harvest
low concentration of extracted DNA/RNA	too much **Elution Buffer** incorrect storage of starting material	elute the DNA/RNA twice with lower volume of **Elution Buffer** ensure that the storage of starting material was correct; avoid thawing of the material
DNA/RNA does not perform well in downstream-applications (e.g. RT-PCR or PCR)	ethanol carry-over during elution salt carry-over during elution	increase g-force or centrifugation time when drying the **Spin Filter** ensure that the **Wash Buffers** are at room temperature check the **Wash Buffers** for salt precipitates. If there are any precipitates, dissolve these precipitates by carefully heating up the wash buffers

10. Limitations of the method

1. The RIDA® Xtract kit is validated for human serum, plasma specimen, cerebral fluid, cell culture supernatant, other cell free body fluids (e.g. urine), swabs, tissue biopsies and stool specimen.

2. Fresh or frozen plasma or serum samples can contain anti-coagulants like EDTA or citrate, but not heparin.
3. Frozen serum or plasma samples must not be thawed more than once. Repeated freeze-thawing leads to denaturation and precipitation of proteins, resulting in reduced viral/bacterial titers and therefore reduced yields of viral/bacterial nucleic acids. In addition cryoprecipitate formed during freeze-thawing will clog the Spin Filter membrane.
4. The amount of purified DNA and/or RNA in the RIDA® Xtract kit procedures depend on the sample type, sample source, transport, storage, age and the virus / bacteria titer.
5. Any diagnostic results generated by using this sample preparation procedure in conjunction with any downstream diagnostic assay, should be interpreted with regard to other clinical or laboratory findings.
6. The included kit components are only useable once.
7. Differences in starting material or flow trace may lead to inoperability in downstream applications.
8. The user is responsible to validate the performance of the RIDA® Xtract kit for any particular use.

11. Explanation of symbols

11.1 General symbols

 Manufacturer

 Lot number

 Article number

 Date of manufacture

 Expiry

 Consult instructions for use

 Store at

 do not reuse

 Number of preparations

IVD For *in vitro* diagnostic use

12. Appendix

12.1 General notes on handling DNA

12.1.1 Nature of DNA

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure good performance in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern Blotting, long-template PCR, and construction of cosmid libraries.

12.1.2 Handling fresh and stored material before the extraction of DNA

For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been snap frozen in liquid nitrogen and stored at - 70 °C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

12.1.3 Storage of DNA

Store DNA at 2 - 8 °C. Storing genomic DNA at -15 – -25 °C can cause shearing of DNA, particularly, if the DNA is exposed to repeated freeze-thaw cycles. Plasmid DNA and other small circular DNAs can be stored at 2 - 8 °C or at -15 – -25 °C.

12.1.4 Drying, dissolving and pipetting DNA

Avoid overdrying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution. Plasmid DNA and other small circular DNAs can be vacuum-dried.
To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively, leave the DNA in buffer overnight at 2 - 8 °C. Minimize vortexing of genomic DNA since this can cause shearing.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA. Regular pipette tips pose no problem for plasmid DNA and other small ring-like DNA structures.

12.1.5 Quantification

Quantification of DNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. The included Carrier Nucleic Acids as well as DNA or RNA, which is co-purified, will interfere with other methods.

12.2 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 have 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 4 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 minutes 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 be allowed to dry.
- non-disposable plasticware should be treated before use to ensure that it is RNase free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- all buffers must be prepared from DEPC-treated RNase free ddH_2O.
- change gloves frequently and keep tubes closed.
- 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

This kit should only be used by personnel trained in *in vitro* diagnostic laboratory practice.

12.2.1 Storage of RNA

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

12.2.2. Quantification

Quantification of DNA and RNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. The included Carrier Nucleic Acids as well as DNA or RNA, which is co-purified, will interfere with other methods.

12.3. Carrier RNA

Carrier RNA serves two purposes. Firstly, it enhances the binding of viral nucleic acids to the Spin Filter membrane, especially if there are very few target molecules in the sample. Secondly, the addition of large amounts of Carrier RNA reduces the chance of viral nucleic acid degradation (in the rare event that RNase or DNase molecules are not denaturated by the salts and detergents in the Lysis Buffer in the Reaction Tube).

12.4. Yield and quality of pathogen DNA/RNA

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates from this kit contain both pathogen DNA and Carrier RNA, and amounts of Carrier RNA will greatly exceed amounts of pathogen nucleic acids.

Yields of pathogen DNA or RNA isolated from biological samples are normally less than 1 µg and therefore difficult to determine photometrically.[1] Keep in mind that the Carrier RNA (5 µg per 200 µl sample) will account for most of the DNA present.

Systems, which isolate simultaneously DNA and RNA, use buffers adapted for the binding of DNA and RNA. As the optimal binding conditions of RNA and DNA are different, such solutions can show a little reduced sensitivity in comparison to kits optimized only to DNA or RNA isolation

[1] In Gel Electrophoresis and in Capillary Electrophoresis, DNA extracted with the RIDA® Xtract kit appears to be degraded because the kit contains Carrier RNA. This is poly A RNA in fragments of 100 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.

12.5 Downstream Applications

Due to the high purity, the isolated viral DNA/RNA and bacterial DNA is ready to use for a broad panel of downstream applications:

- real-time (RT)-PCR (e.g. TaqMan)
- cDNA synthesis
- microarray-application
- RFLP-Analysis