Instructions for Use

Life Science Kits & Assays

innuPREP DNA/RNA Mini Kit

Order No.:

845-KS-2080010	10 reactions
845-KS-2080050	50 reactions
845-KS-2080250	250 reactions

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It needs not necessarily agree with future versions. Subject to change!

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1. Introduction

1.1 Intended use

The innuPREP DNA/RNA Mini Kit has been designed for the extraction of DNA and RNA from eukaryotic cells, tissue samples and bacteria. The kit uses an optimized chemistry resulting in a fast and reliable purification of RNA with high quality and yield.

1.2 Notes on the use of this manual and the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

Symbol	Information
REF	**REF** Catalogue number
Σ N	**Content** Contains sufficient reagents for <N> reactions
15 °C 30 °C	**Storage conditions** Store at room temperature or shown conditions respectively
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge
	Manufactured by Contact information of manufacturer
	For single use only Do not use components for a second time
	Note / Attention Observe the notes marked in this way to ensure correct function of the kit and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual" p. 2).
- Working steps are numbered.

2. Safety precautions

NOTE

Read through this chapter carefully before guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!

This kit is made for single use only!

ATTENTION!

Don't eat or drink components of the kit!
The kit shall only be handled by educated personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. IST Innuscreen GmbH has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely, but cannot be excluded completely. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!

Do not add bleach or acidic components to the waste after sample preparation!

NOTE

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany
Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3. General notes and safety recommendations 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 to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)

- Non-disposable plastic ware 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 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 inactivate many RNases completely. Oven baking inactivates RNases and ensures 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 has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C and then it has to 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, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free ddH_2O.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

4. Storage conditions

The Kit is shipped at ambient temperature. Upon arrival the **innuPREP DNA/RNA Mini Kit** should be stored dry, at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

If there are any precipitates within the provided solutions solve these precipitates by careful warming. Before every use make sure that all components have room temperature.

5. Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the innuPREP DNA/RNA Mini Kit or other IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 30 9486 9980. For other countries please contact your local distributor.

6. Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ "Product specifications" p. 9). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE

The kit is for research use only!

7. Kit components

7.1 Included kit components

	Σ 10	Σ 50	Σ 250
REF	845-KS-2080010	845-KS-2080050	845-KS-2080250
Lysis Solution RL	6 ml	30 ml	125 ml
Washing Solution HS (conc.)	6 ml	30 ml	2 x 70 ml
Washing Solution LS (conc.)	3 ml	2 x 8 ml	2 x 40 ml
RNase-free Water	2 ml	6 ml	25 ml
Elution Buffer	2 ml	6 ml	30 ml
Spin Filter D	10	50	5 x 50
Spin Filter R	10	50	5 x 50
Receiver Tubes	40	4 x 50	20 x 50
Elution Tubes	20	2 x 50	10 x 50
Manual	1	1	1

7.2 Components not included in the kit

- 1.5 ml reaction tubes
- 70 % and 96–99.8 % ethanol, non denatured or methylated

For Protocol 3:

- TE-Buffer (10 mM Tris-HCl; 1 mM EDTA; pH 8.0)
- Lysozyme (10 mg/ml, 400 U/µl), Mutanolysin (0.4 U/µl), Lysostaphin (0.4 U/µl) **OR** innuPREP Bacteria Lysis Booster (IST Innuscreen GmbH, 845-KA-1000050)

8. Product specifications

1. Starting material:

- up to 5 x 10^6 eukaryotic cells
- up to 20 mg tissue samples
- up to 1 x 10^9 bacterial cells (Gram-positive & Gram-negative bacteria)

NOTE
Avoid freezing and thawing of starting material.

2. Time for isolation:

   Approximately 15-40 min

3. Typical yield: depends on the kind and initial amount of the starting material

9. Initial steps before starting

- Add the indicated amount of absolute ethanol to each bottle **Washing Solution HS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-2080010	Add 6 ml ethanol to 6 ml Washing Solution HS (conc.).
845-KS-2080050	Add 30 ml ethanol to 30 ml Washing Solution HS (conc.).
845-KS-2080250	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).

- Add the indicated amount of absolute ethanol to each bottle **Washing Solution LS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-2080010	Add 12 ml ethanol to 3 ml Washing Solution LS (conc.).
845-KS-2080050	Add 32 ml ethanol to 8 ml Washing Solution LS (conc.).
845-KS-2080250	Add 160 ml ethanol to 40 ml Washing Solution LS (conc.).

- Centrifugation steps Centrifugation steps should be carried out at room temperature.

10. Protocols for isolation of RNA and DNA

10.1 Protocol 1: extraction from tissue samples

10.1.1 Homogenization of starting material

For the homogenization of tissue sample it is possible to use commercially available rotor-stator homogenizer or bead mills. It is also possible to disrupt the starting material using mortar and pestle in liquid nitrogen and grind the tissue sample to a fine powder.

NOTE
To maximize the final yield of total RNA a complete homogenization of tissue sample is important! Avoid freezing and thawing of the sample.

A Homogenization using a rotor-stator homogenizer or bead mills

1. Weigh up to 20 mg of fresh or frozen starting material (tissue, biopsie) and transfer it in a suitable reaction vessel for the homogenizer or in a vessel pre-filled with beads.
2. Add **450 µl Lysis Solution RL.**
3. Homogenize the sample.
4. Transfer the homogenized tissue sample into a 1.5 ml reaction tube and place the sample under **Lysis Solution RL** for longer storage at -22 °C to -18 °C or continue with 10.1.2

B Disruption using a mortar and pestle

1. Weigh up to 20 mg fresh or frozen starting material (tissue, biopsie) in a pre-cooled mortar. Grind the material under liquid nitrogen with a pestle to a fine powder.
2. Transfer the powder into a 1.5 ml reaction tube. Don’t allow the sample to thaw!
3. Add **450 µl Lysis Solution RL** and incubate the sample for appropriate time for a further lysis under continuous shaking.
4. Finally place the sample under Lysis Solution RL for longer storage at −22 °C to -18 °C or continue with 10.1.2.

10.1.2 Extraction of RNA and DNA

1. Spin down unlysed material by centrifugation at maximum speed for 1 minute. Place a Spin Filter D into a Receiver Tube. Transfer the supernatant of the lysed sample onto the Spin Filter D. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.

ATTENTION
Do not discard the filtrate, because the filtrate contains the RNA!

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

2. Place the Spin Filter D into a new Receiver Tube. The genomic DNA is bound onto Spin Filter D. Processing of the Spin Filter D will be continued after binding of total RNA onto Spin Filter R.

3. Place a Spin Filter R into a new Receiver Tube. Add an **equal volume** (appr. 400 µl) of **70 % ethanol** to the filtrate from step 1. Mix the sample by pipetting up and down several times. Transfer the sample onto the Spin Filter R. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

4. Discard the Receiver Tube with filtrate.

5. Place the Spin Filter R into a new Receiver Tube. The total RNA is bound on Spin Filter R. Both Spin Filters (Spin Filter D and R) will be processed in parallel now.

6. Open the Spin Filters D and R, add **500 µl Washing Solution HS** to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.

7. Open the Spin Filters D and R, add **700 µl Washing Solution LS** to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for

1 minute. Discard the filtrate and re-use the Receiver Tubes. Place the Spin Filters D and R again into the Receiver Tubes.

8. Centrifuge at 10,000 x g (12,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tubes.

9. Place the Spin Filters D and R each into an Elution Tube. Carefully open the caps of the Spin Filters D and R, add **100 µl Elution Buffer** to Spin Filter D and **30–80 µl RNase-free Water** to Spin Filter R. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (~8,000 rpm) for 1 minute.

NOTE

Depending on the extracted yield or the needed concentration of genomic DNA or total RNA you can also elute with different volumes of Elution Buffer/RNase-free water. A lower volume of Elution Buffer/RNase-free Water increases the concentration of DNA/RNA and a higher volume of Elution Buffer/RNase-free Water leads to an increased yield but a lower concentration of nucleic acids. Please note, that the minimum of RNase-free water should be 20 µl. Store nucleic acids at appropriate conditions (RNA at −80 °C and DNA at −20 °C)!

10.2 Protocol 2: extraction from eucaryotic cells

1. Transfer up to 5 x 10^6 eukaryotic cells into a tube 2.0 ml tube and pellet the cells by centrifugation with parameters adequate for the cell type (e.g. 2-5 minutes at 5,000 x g). Discard the supernatant. Do not discard the pellet!

2. Add **400 µl Lysis Solution RL** to the cell pellet. Incubate for 2 minutes at room temperature. Re-suspend the cell pellet completely by pipetting up and down. Incubate the sample for further 3 minutes at room temperature.

NOTE

To maximize the final yield of DNA and total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step.

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3. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute. Place a Spin Filter D into a Receiver Tube. Transfer the supernatant of the lysed sample onto the Spin Filter D. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.

ATTENTION

Do not discard the filtrate, because the filtrate contains the RNA!

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

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4. Place the Spin Filter D into a new Receiver Tube. The genomic DNA is bound onto Spin Filter D. Processing of the Spin Filter D will be continued after binding of total RNA onto Spin Filter R.

5. Place a Spin Filter R into a new Receiver Tube. Add an equal volume (appr. 400 µl) of 70 % ethanol to the filtrate from step 3. Mix the sample by pipetting sometimes up and down. Transfer the sample onto the Spin Filter R. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

6. Discard the Receiver Tube with filtrate.
7. Place the Spin Filter R into a new Receiver Tube. The total RNA is bound on Spin Filter R. Both Spin Filters (Spin Filter D and R) will be processed in parallel now.
8. Open the Spin Filters D and R, add 500 µl Washing Solution HS to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.
9. Open the Spin Filters D and R, add 700 µl Washing Solution LS to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.
10. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tubes.
11. Place the Spin Filters D and R each into an Elution Tube. Carefully open the caps of the Spin Filters D and R, add 100 µl Elution Buffer to Spin Filter D and 30–80 µl RNase-free Water to Spin Filter R. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (~8,000 rpm) for 1 minute.

NOTE

Depending on the extracted yield or the needed concentration of genomic DNA or total RNA you can also elute with different volumes of Elution Buffer/RNase-free Water. A lower volume of Elution Buffer/RNase-free Water increases the concentration of DNA/RNA and a higher volume of Elution Buffer/RNase-free Water leads to an increased yield but a lower concentration of nucleic acids. Please note, that the minimum of RNase-free water should be 20 µl. Store nucleic acids at appropriate conditions (RNA at −80 °C and DNA at −20 °C)!

10.3 Protocol 3: extraction from bacterial cells

I. Pre-lysis of bacterial cell walls

1. Transfer bacterial culture (volume depends on the concentration of starting material) into a tube 2.0 ml tube.
2. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 2-5 minutes at 5,000 x g (~7.500 rpm)). Discard the supernatant. Do not discard the pellet!
3. Resuspend the bacterial cell pellet in **100 µl TE-Buffer**.

A Gram-negative bacteria

NOTE
Although Gram-negative bacteria do not require a pre-lysis step, using Lysozyme (not included in the kit) can enhance the efficiency of lysis.

4. Add **20 µl Lysozyme** (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
5. Proceed with step II p. 15.

B Gram-positive bacteria

NOTE
Gram-positive bacteria require a pre-lysis step using Mutanolysin and/or Lysozyme (not included in the kit).

4. Add **20 µl Lysozyme** (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
5. Add **5 µl Mutanolysin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
6. Proceed with step II p. 15.

NOTE
Lysozyme and Mutanolysin exert synergistic activity. Using both enzymes together will increase the yield of isolated nucleic acids.

C Staphylococcus strains

NOTE
For pre-lysis of *Staphylococcus* the enzyme Lysostaphin is recommended (not included in the kit).

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4. Add **10 µl Lysostaphin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
5. Proceed with step II p. 15.

D Alternative pre-lysis using innuPREP Bacteria Lysis Booster

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NOTE
The innuPREP Bacteria Lysis Booster Kit has been developed for a highly efficient pre-lysis of bacterial cell walls by generating spheroblasts. This new mixture of different enzymes boosts the lysis of all bacteria in particular the hard-to-lyse microorganisms like *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Bacillus* and *Clostridium*.

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4. Add **70 µl TE-Buffer** to the resuspended cell pellet and mix again.
5. Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.
6. Add **12 µl enzyme mix** to the sample and vortex shortly. Incubate the sample for 30 minutes at 37 °C.
7. Proceed with step II p. 15.

II. Isolation of RNA and DNA

1. Add 450 µl Lysis Solution RL to the sample and vortex vigorously or pipette sometimes up and down. Incubate the sample for further 3 minutes at room temperature.

NOTE
To maximize the final yield of DNA and total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step.

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2. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute. Place a Spin Filter D into a Receiver Tube. Transfer the supernatant of the lysed sample onto the Spin Filter D. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.

ATTENTION
Do not discard the filtrate, because the filtrate contains the RNA!

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

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3. Place the Spin Filter D into a new Receiver Tube. The genomic DNA is bound onto Spin Filter D. Processing of the Spin Filter D will be continued after binding of total RNA onto Spin Filter R.

4. Place a Spin Filter R into a new Receiver Tube. Add an equal volume (appr. 600 µl) of 70 % ethanol to the filtrate from previous step 2. Mix the sample by pipetting sometimes up and down.

5. Transfer 650 µl of the sample onto the Spin Filter R. Centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter R back into the Receiver Tube. Load the residual sample on the Spin Filter R and centrifuge again at 10,000 x g (~12,000 rpm) for 1 minute.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

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6. Discard the filtrate and re-use the Receiver Tube. The total RNA is bound onto Spin Filter R. Both Spin Filters (Spin Filter D and R) will be processed in parallel now.

7. Open the Spin Filters D and R, add **500 µl Washing Solution HS** to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.

8. Open the Spin Filters D and R, add **700 µl Washing Solution LS** to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.

9. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tubes.

10. Place the Spin Filter D and R each into an Elution Tube. Carefully open the caps of the Spin Filters D and R, add **100 µl Elution Buffer** to Spin Filter D and **30–80 µl RNase-free Water** to Spin Filter R. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (~8,000 rpm) for 1 minute.

NOTE

Depending on the extracted yield or the needed concentration of genomic DNA or total RNA you can also elute with different volumes of Elution Buffer/RNase-free Water. A lower volume of Elution Buffer/RNase-free Water increases the concentration of DNA/RNA and a higher volume of Elution Buffer/RNase-free Water leads to an increased yield but a lower concentration of nucleic acids. Please note, that the minimum of RNase-free Water should be 20 µl. Store nucleic acids at appropriate conditions (RNA at −80 °C and DNA at −20 °C)!

11. Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient disruption or homogenization	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no DNA or total RNA eluted	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer and RNase-free water to 5 minutes or repeat elution step once again.
DNA contamination of extracted RNA	
Too much starting material	Reduce amount of starting material.
Incorrect lysis of starting material	Use the recommended techniques for lysis of cell pellet. Perform DNase digest of the eluate containing the total RNA or perform a on column DNase digest step after binding of the RNA on Spin Filter R!
Total RNA degraded	
RNA source inappropriately handled or stored	Ensure that the starting material is fresh! Ensure that the protocol, especially the first steps, has been performed quickly.
RNase contamination of solutions; Receiver Tubes, etc.	Use sterile, RNase-free filter tips. Before every preparation clean up the pipette, the devices and the working place. Always wear gloves!
Total RNA does not perform well in downstream applications (e.g. RT-PCR)	
Ethanol carryover during elution	Increase time for removing of ethanol.
Salt carryover during elution	Ensure that Washing Solution HS and Washing Solution LS are at room temperature. Check up Washing Solution for salt precipitates. If there are any precipitate dissolves these precipitate by carefully warming.

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