foodproof® Sample Preparation Kit IV

Revision B, December 2025

For the preparation of viral RNA from cell-free biological samples.

Product No. KIT230185

Kit for 50 isolations

Store Component A at 15 to 25 °C

Store Component B at -15 to -25 °C

Store Component C at 15 to 25 °C

For food testing purposes.

FOR *IN VITRO* USE ONLY

Table of Contents

1. What this Product Does

The foodproof Sample Preparation Kit IV is optimized for the isolation of viral RNA from various food samples (raw material and processed food) and water samples.

1.1 Number of Preparations

50 isolations.

1.2 Storage and Stability

The foodproof Sample Preparation Kit IV Component B must be stored frozen at -15 to -25 °C.
Components A and C must be stored at 15 to 25 °C.
Kit components are guaranteed to be stable until the expiration dates printed on the labels.
***Note*:** Improper storage at 2 to 8 °C (refrigerator) or -15 to -25 °C (freezer) will adversely impact RNA purification when precipitates form in the solutions.

1.3 Kit Contents

All solutions are clear and should not be used when precipitates have formed. If precipitates have formed, simply warm the solutions at 15 to 25 °C or in a 37 °C water bath until the precipitates have dissolved.

Vial / Cap Color	Label	Contents / Function
Component A (Storage conditions: protected from light at 15 to 25 °C)		
1 red	foodproof Sample Preparation Kit IV - Lysis Buffer	• 2 x 50 mL • For lysis of virus particles and extraction of RNA
2 colorless	foodproof Sample Preparation Kit IV - Wash Buffer I	• 13 mL, add 17 mL absolute ethanol • For removing impurities
3 blue	foodproof Sample Preparation Kit IV - Wash Buffer II	• 9 mL, add 21 mL absolute ethanol • For removing impurities
Component B (Storage conditions: -15 to -25 °C)		
4 colorless	foodproof Sample Preparation Kit IV - Elution Buffer	• 3 x 1.25 mL • For elution of RNA • Contains Ribonuclease Inhibitor
5 purple	foodproof Sample Preparation Kit IV - Carrier-tRNA	• 1 x 600 µL • For the enhancement of viral RNA binding to the fiberglass fleece
Component C (Storage conditions: dry at 15 to 25 °C)		
6	Filter Tubes	• Bag with 50 polypropylene tubes with two layers of fiberglass fleece, for use with up to 700 µL sample volume
7	Collection Tubes	• 9 x Bags with 50 polypropylene tubes (2 mL)

1.4 Chemical Hazard

The Lysis Buffer (vial 1) and the Wash Buffer I (vial 2) contain irritating compounds that are harmful when brought into contact with skin, inhaled, or swallowed. Always store and use the buffers away from food for humans and animals. Always wear gloves and follow standard safety precautions during handling.

1.5 Additional Equipment and Reagents Required

- Ethanol, absolute
- Water, double-distilled
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force (e.g., Eppendorf 5424 or equivalent)
- Vortex

2. How to Use this Product

2.1 Test Principle

The viral RNA is released during the lysis of the virus particles by a short incubation period with the provided Lysis Buffer. The added carrier-tRNA enhances RNA binding to the fiberglass fleece and thus, increases the sensitivity of the sample preparation. After removing the hydration shell from the RNA by adding absolute ethanol to the sample, the RNA selectively binds to special glass fibers pre-packed in the filter tube. Bound RNA is purified in two “wash-and-spin” steps to remove potential PCR inhibitors, and then a low-salt elution releases the RNA from the glass fiber. This simple method eliminates the need for organic-solvent extractions and RNA precipitation, thus providing rapid, simultaneous purification of many samples.

2.2 Basic Steps

Stage	Description
1	RNA is extracted by incubation with the Lysis Buffer and carrier-tRNA.
2	Absolute ethanol removes the hydration shell from RNA.
3	RNA is bound to the glass fibers, pre-packed in the filter tube.
4	Washing of bound RNA and purification from salts, proteins, and other cellular impurities.
5	Purified RNA is recovered using the Elution Buffer and stabilized by the ribonuclease inhibitor.

2.3 Application

Depending on the food, different virus pre-concentration and purification steps may be necessary. The protocols for the concentration and purification of the noroviruses and hepatitis A viruses from soft fruits, bivalve molluscan shellfish, and bottled water are all listed in detail by the ISO/TS 15216-1:2013: Microbiology of food and animal feed -- Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR. Hygiena Diagnostics recommends the use of these protocols with this kit prior to RNA extraction.

The quality of the RNA obtained with the kit is highly suitable for qualitative and quantitative applications using any reverse transcriptase-PCR (RT-PCR) system.

Note: The recommended amplicon length for RT-PCR analysis is < 200 bp because the RNA of processed food may be degraded to a certain extent.

2.4 Sample Material

Use 140 µL or 500 µL cell-free biological material. Other volumes can be calculated by multiplying the amount of Lysis Buffer, absolute ethanol, and Carrier-tRNA by a factor of 4 (i.e., x 4).

Note: Higher sample volumes (over 500 µL) reduce the number of possible preparations, since higher volumes of Lysis Buffer, absolute Ethanol, and Carrier-tRNA are needed. Additional aliquots can be prepared and the RNA can be concentrated by loading them step-by-step into one Filter Tube. This procedure is recommended if the RNA content of the sample is low.

2.5 Quality Control

- MS2-phage (10 – 10,000 PFU) is used for quality check.
  - 10 µL of the eluate is analyzed using the foodproof Norovirus Detection Kit (GI, GII) (Product No. KIT230055). Specific amplification signals were obtained.
  - The absence of contaminating RNA was controlled by testing an additional RNA preparation and a subsequent PCR test with molecular-grade water. No amplification product was detected.

3. Procedures and Required Materials

3.1 Before You Begin

3.1.1 Preparation of Kit Working Solutions

In addition to the ready-to-use solutions supplied with the kit, you will need the following working solutions. Preparation of working solutions is required.

Bottle / Cap Color	Content	Preparation of Working Solution	Storage and Stability
2 colorless	foodproof Sample Preparation Kit IV - Wash Buffer I	Add 17 mL absolute ethanol to Wash Buffer I. ***Note:*** Label and date bottle after ethanol is added.	Store at 15 to 25 °C Stable until the expiration date printed on the kit label.
3 blue	foodproof Sample Preparation Kit IV - Wash Buffer II	Add 21 mL absolute ethanol to Wash Buffer II. ***Note:*** Label and date bottle after ethanol is added.	Store at 15 to 25 °C Stable until the expiration date printed on the kit label.

3.1.2 Virus isolation and concentration

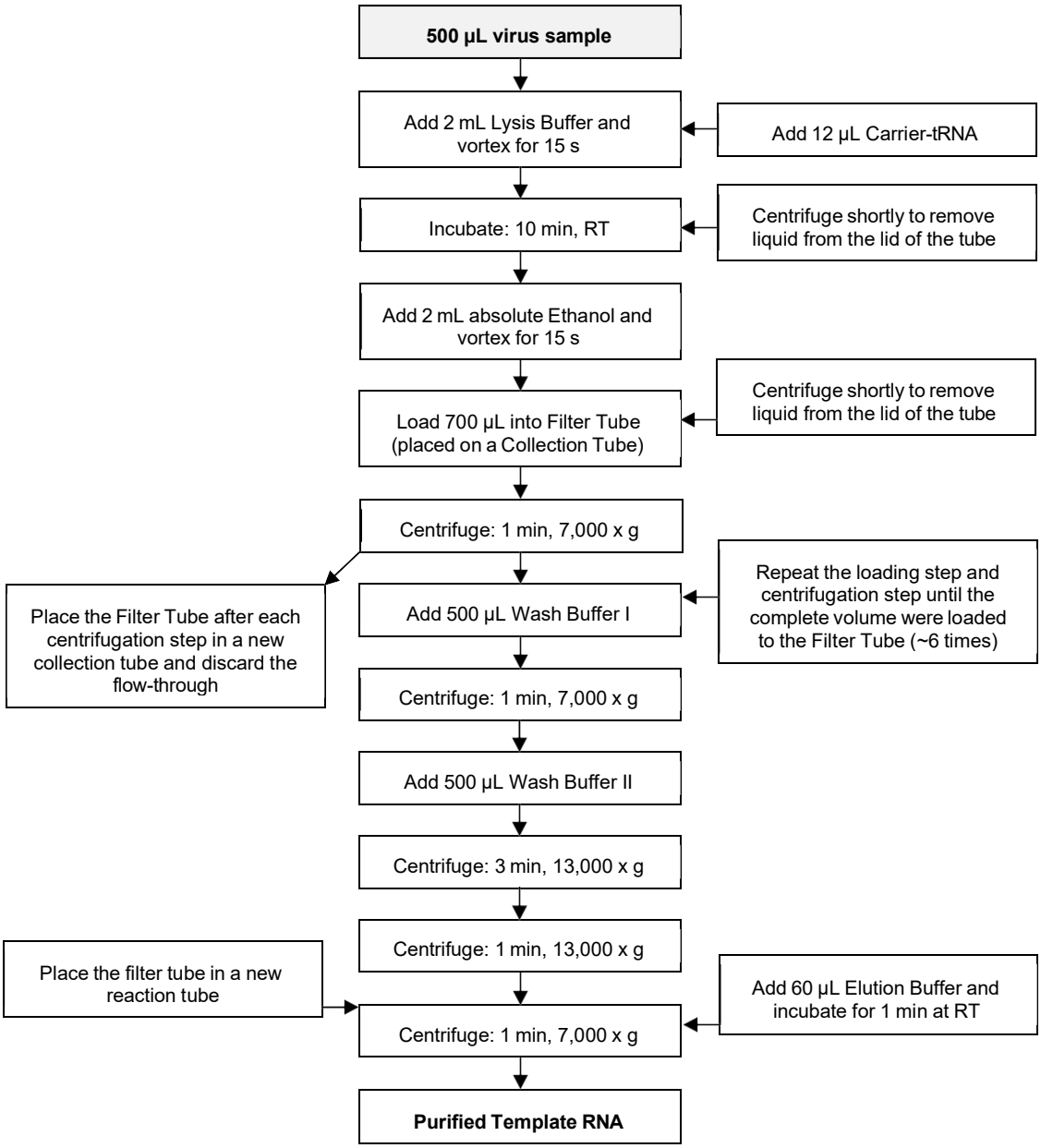
The foodproof Sample Preparation Kit IV is for RNA preparation from cell-free biological samples. Therefore, food and water samples must be pre-concentrated by following the protocols of ISO/TS 15216-2:2013 (see references) or other approved methods before the RNA can be isolated with this kit. The following table provides a short overview of the applied methods:

Material	Method	RNA preparation protocol
Soft fruits and salad	Virus Elution followed by PEG-precipitation; purification with chloroform:butanol	Initial sample volume 500 µL
Mussels	Proteinase-K digestion of the intestine	Initial sample volume 500 µL
Minced meat	Proteinase-K digestion and ultrafiltration	Initial sample volume 140 µL
Bottled water	Filtration	Initial sample volume 500 µL
Surfaces	Swab samples are squeezed in the Lysis Buffer	Initial sample volume 140 µL
Stool	Resuspension of stool at a final 10% stool:PBS (w/v) concentration	Initial sample volume 140 µL

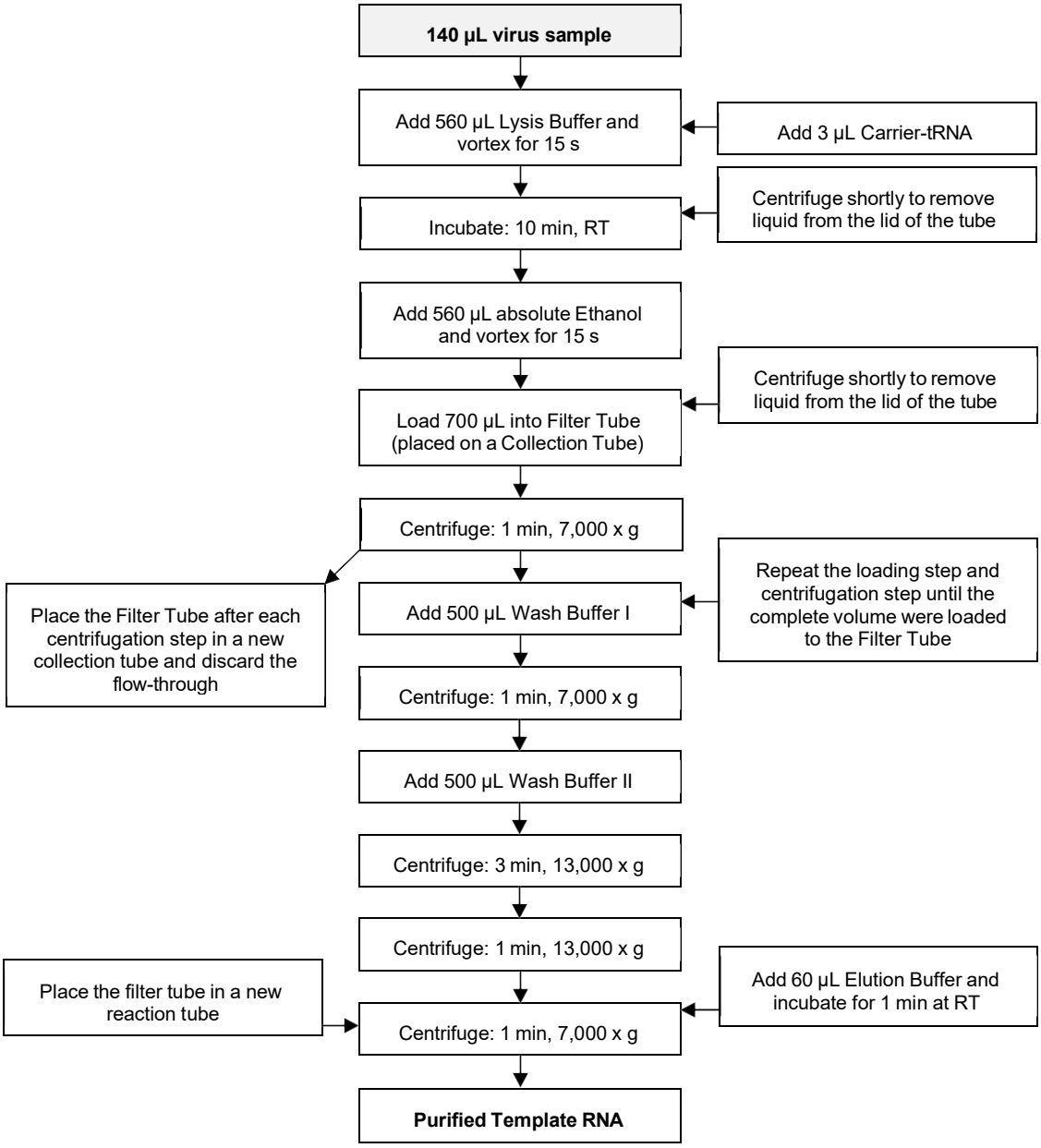
Caution

Use sterile disposable polypropylene tubes and aerosol filter tips to avoid cross-contamination. Always wear gloves during the assay and follow safety precautions to minimize contact when handling.

3.2 Flow Chart: 500 µL sample



3.3 Flow Chart: 140 µL sample



3.4 Isolation Procedure

Caution

RNA is far less stable when compared to DNA. RNA-degrading enzymes (RNases) are permanently present in samples and lab materials. Surfaces and instruments (e.g., pipettes) should be sterilized with an appropriate disinfectant before you begin work to achieve satisfactory results. Use sterile disposable polypropylene tubes and filter tips in order to avoid cross-contamination. Always wear gloves during the assay and follow safety precautions to minimize contact when handling.

3.4.1 Procedure A for 500 µL initial sample volume

Note: Fill up your sample volume with molecular-grade water or PBS to 500 µL, if you have a smaller volume/amount.

Step	Action	Volume	Time/g Time/Temp.
1	• Add 500 µL virus sample to 2 mL Lysis Buffer (bottle 1, red cap) containing 12 µL Carrier-tRNA (bottle 5, purple cap) in a 13-15 mL reaction tube • Vortex for 15 s • Incubate: 10 min, RT • Centrifuge shortly to remove the liquid from the lid of the tube	2 mL 12 µL	10 min, RT 10 s at 7,000 x g
2	• Add 2 mL absolute ethanol (not provided) • Vortex for 15 s • Centrifuge shortly to remove the liquid from the lid of the tube	2 mL	10 s at 7,000 x g
3	• Pipet the 700 µL volume into the upper reservoir of a combined filter tube collection tube assembly	700 µL	
4	• Centrifuge		1 min at 7,000 x g
5	• Place the filter tube in a new collection tube and discard the flow-through • Repeat steps 3 and 4 until the complete volume per sample is loaded on a filter tube	700 µL	
6	• Place the filter tube in a new collection tube and discard the flow-through • Add 500 µL of the Wash buffer I (bottle 2, colorless cap)	500 µL	
7	• Centrifuge		1 min at 7,000 x g
8	• Place the filter tube in a new collection tube and discard the flow-through • Add 500 µL of the Wash buffer II (bottle 3, blue cap)	500 µL	
9	• Centrifuge		3 min at 13,000 x g
10	• Place the filter tube in a new collection tube and discard the flow-through		
11	• Centrifuge		1 min at 13,000 x g

12	• Insert filter tube in a clean 1.5 mL reaction tube (not provided) • Add 60 µL Elution Buffer (vial 4, colorless cap) in the middle of the glass fiber (do not touch the glass fiber!) • Incubate for 1 min at RT	60 µL	15 - 25 °C for 1 min
13	• Centrifuge		1 min at 7,000 x g
14	The microcentrifuge tube now contains the eluted RNA.		

3.4.2 Procedure B for 140 µL initial sample volume

Note: Bring total sample volume to 140 µL using molecular-grade water or PBS.

Step	Action	Volume	Time/g Time/Temp.
1	• Add 140 µL virus sample to 560 µL Lysis Buffer (bottle 1, red cap) containing 3µL Carrier-tRNA (bottle 5, purple cap) in a 2 mL reaction tube • Vortex for 15 seconds • Incubate: 10 min, RT • Centrifuge shortly to remove the liquid from the lid of the tube	560 µL 3 µL	10 min, RT 10 s at 7,000 x g
2	• Add 560 µL absolute ethanol (not provided) • Vortex for 15 seconds • Centrifuge shortly to remove the liquid from the lid of the tube	560 µL	10 s at 7,000 x g
3	• Pipette the 700 µL volume into the upper reservoir of a combined filter tube collection tube assembly	700 µL	
4	• Centrifuge		1 min at 7,000 x g
5	• Place the filter tube in a new collection tube; discard the flow-through • Repeat steps 3 and 4 until the complete volume per sample is loaded on a filter tube	700 µL	
6	• Place the filter tube in a new collection tube; discard the flow-through • Add 500 µL of the Wash buffer I (bottle 2, colorless cap)	500 µL	
7	• Centrifuge		1 min at 7,000 x g
8	• Place the filter tube in a new collection tube and discard the flow-through • Add 500 µL of the Wash buffer II (bottle 3, blue cap)	500 µL	
9	• Centrifuge		3 min at 13,000 x g
10	• Place the filter tube in a new collection tube and discard the flow-through		
11	• Centrifuge		1 min at 13,000 x g

12	• Insert filter tube in a clean 1.5 ml reaction tube (not provided) • Add 60 µL Elution Buffer (vial 4, colorless cap) in the middle of the glass fiber (do not touch the glass fiber!) • Incubate for 1 min at RT (15 to 25 °C)	60 µL	15 - 25 °C for 1 min
13	• Centrifuge		1 min at 7,000 x g
14	The microcentrifuge tube now contains the eluted RNA.		

3.5 Storage of Samples

IF You Want to …	THEN …
Continue analysis	Use the eluted RNA directly. (We recommend using the RNA immediately for RT-PCR experiments, if possible.)
Stop	Do not store the RNA longer than 60 min at 4°C. Store the RNA at -20 °C for short-term periods (1 day). Store the RNA at -80 °C for long-term storage.

4. Appendix

4.1 Troubleshooting

Problem	Possible Cause	Recommendation
Low RNA yield or purity	Kit stored under non-optimal conditions.	• Store the kit protected from light at 15 to 25 °C at all times upon arrival. • Store the Elution Buffer and the Carrier-tRNA at -15 to -25 °C.
	Buffers or other reagents were exposed to conditions that reduce their effectiveness.	• Store all buffers at 15 to 25 °C protected from light, except for Elution Buffer, which must be stored at -15 to -25 °C. • Store Carrier-tRNA at -15 to -25 °C and prepare aliquots to avoid frequent freeze-thaw cycles. • Close all reagent bottles tightly after each use to preserve pH and stability, and to prevent contamination.
	Ethanol not added to the Wash Buffers I and II.	• Add absolute ethanol to Wash Buffer I and II before use. • After adding ethanol, mix the Wash Buffer I and II well, and store at 15 to 25 °C protected from light. • Always mark Wash Buffer bottles to indicate the addition of ethanol.
	Reagents and samples not completely mixed.	• Always mix the sample tube well after addition of each reagent.
	Loss of virus material during pre-concentration steps before RNA preparation starts	• Very low recovery rates have been reported in literature for virus concentration methods from food, especially for soft fruits (~90 -99.9%). However, the loss of virus varies between sub-samples. Repetition or a higher sub-sample size may overcome these problems.
Absorbency (A_{260}) reading of product too high	Glass fibers that can coelute with RNA scatter light.	• After elution step is complete, remove filter from tube containing eluted sample and spin sample tube for 2 min at maximum speed. • Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Sample “pops” out of wells in agarose gels	Eluate containing the purified RNA product is contaminated with ethanol from the Wash Buffers.	• After the last wash step, ensure the flow-through containing Wash Buffer does not contact the bottom of the filter tube. • If this has occurred, empty collection tube, re-insert the contaminated filter tube, and re-centrifuge for 30 s.

4.2 Reference

1. Microbiology of food and animal feed - Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR - Part 2: Method for qualitative detection (ISO/TS 15216-2:2013); German version CEN ISO/TS 15216-2:2013.

5. Supplementary Information

5.1 Ordering Information

Hygiena Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.hygiena.com.

5.2 Trademarks

foodproof® is a registered trademark of Hygiena® Diagnostics GmbH. Other brand or product names are trademarks of their respective holders.

5.3 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, please contact our Technical Support staff (www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

5.4 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 16

6. Change Index

Version 1, December 2016
First version of the package insert.

Version 2, May 2017
Procedure A and B: Incubation step 2 deleted.
Procedure A: 2 mL tube changed to 13-15 mL.

Revision A, January 2024
Rebranding and new layout.
S 400 16 20 -> INS-KIT230185-RevA

Revision B, December 2025
Cap color change in kit, reformatting.
S 400 16 20 -> INS-KIT230185-RevB

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