GenUP™ Virus RNA Kit

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ Virus RNA Kit		
CAT. NO.	BR0701001	BR0701002	BR0701003
SIZE	10 preps	50 preps	250 preps
COMPONENTS			
Buffer LYSIS LR	15 ml	30 ml	160 ml
Buffer BINDING BR	16 ml	40 ml	180 ml
CARRIER (lyophilized)	1 vial (add 1.25 ml Water, RNase-free)	1 vial (add 1.25 ml Water, RNase-free)	3 vials (add 1.25 ml Water, RNase-free)
Water, RNase-free (for CARRIER)	2 ml	2 ml	3 × 2 ml
Proteinase K (lyophilized)	1 vial (add 0.3 ml water)	1 vial (add 1.5 ml water)	4 vials (add 1.5 ml water)
Buffer WASH A (concentrate)	5 ml (add 5 ml ethanol)	15 ml (add 15 ml ethanol)	70 ml (add 70 ml ethanol)
Buffer WASH B (concentrate)	6 ml (add 24 ml ethanol)	16 ml (add 64 ml ethanol)	36 ml (add 144 ml ethanol)
Water, RNase-free (for ELUTION)	2 ml	3 × 2 ml	25 ml
Mini Filters (bordeaux)	10	50	5 × 50
Collection Tubes (2 ml)	50	5 × 50	25 × 50
Elution Tubes (1.5 ml)	10	50	5 × 50

STORAGE

Room temperature (until expiry date – see product label).
If precipitation appears, gently warm the solution to dissolve the precipitate.
Store lyophilized Proteinase K and CARRIER at 4°C,
Store aliquots of dissolved Proteinase K and CARRIER at –20°C.
Store lyophilized and dissolved Carrier RNA at -22 °C to - 18 °C.

FEATURES

- Universal kit for isolating viral RNA from various starting materials
- Fast and simple procedure, flexible elution volumes
- High yields of pure RNA
- No DNase treatment, no toxic β-mercaptoethanol

APPLICATIONS

- Virus RNA isolation from plasma, serum, urine, other body fluids, cell cultures, tissues, and buccal swabs

DESCRIPTION

biotechrabbit™ GenUP Virus RNA Kit has been specially developed for quick and easy isolation of viral RNA. Viral single-stranded RNA can be isolated from eukaryotic samples including plasma, serum, urine, and other body fluids as well as cell cultures, tissues, and buccal swabs.
The unique binding membrane of our high-capacity Mini Filters guaranties high yields. A high concentration of purified RNA can be achieved with flexible elution volumes. The kit includes carrier RNA.
After few initial procedures, the viral RNA is bound to a Mini Filter, washed and then eluted in a separate tube. The purified RNA is ready to be used in all demanding molecular biology applications, including cDNA synthesis, northern blot analysis, qPCR and RT-PCR.

SPECIFICATIONS

STARTING MATERIAL	Eukaryotic cells (up to 5×10^6) Serum, plasma, cell-free body fluids, cell culture supernatants (150 µl) Tissue samples, biopsies (up to 20 mg) Paraffin-embedded tissues, buccal swabs
EXTRACTION TIME	Approximately 25 min
TYPICAL YIELD	Yield is highly dependent on sample type

MATERIALS SUPPLIED BY THE USER

- 96–99.8% ethanol
- Double-distilled water, RNase-free
- PBS (Phosphate buffered saline; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4; pH 7.4) for tissue and biopsy samples
- 0.9% NaCl for swabs
- Centrifugation tubes
- Pipet tips

STEPS BEFORE STARTING

- Add the following volume of 96–99.8% ethanol to each buffer, close firmly, mix thoroughly and store at room temperature.

CAT. NO.		CONCENTRATE	ETHANOL	FINAL VOLUME
Buffer WASH A	BR0701001	5 ml	5 ml	10 ml
	BR0701002	15 ml	15 ml	30 ml
	BR0701003	70 ml	70 ml	140 ml
Buffer WASH B	BR0701001	6 ml	24 ml	30 ml
	BR0701002	16 ml	64 ml	80 ml
	BR0701003	36 ml	144 ml	180 ml

- Add the following volume of double-distilled water, RNase-free to each vial Proteinase K, mix thoroughly and store 0.3 ml aliquots at –20°C. Avoid repeated freezing and thawing.

BR0701001	0.3 ml
BR0701002, BR0701003	1.5 ml for 5 × 0.3 ml aliquots

- Add 1.25 ml Water, RNase-free (for CARRIER), to each vial CARRIER, mix thoroughly and store 0.25 ml aliquots at –20°C. CARRIER contains RNA: avoid contamination with RNases.
- Before each purification, prepare a fresh mix of CARRIER and Buffer LYSIS LR as follows. Store at 4°C and use within one day.

COMPONENT	FOR 5 SAMPLES	FOR 10 SAMPLES	FOR N SAMPLES
Buffer LYSIS LR	2.7 ml	5.4 ml	540 µl × n
CARRIER solution prepared as above	60 µl	120 µl	12 µl × n
Final volume	2.76 ml	5.52 ml	552 µl × n

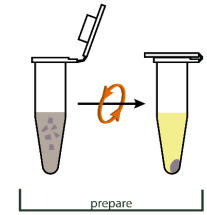
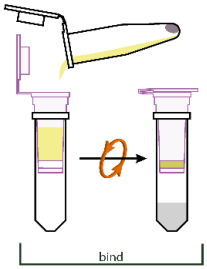
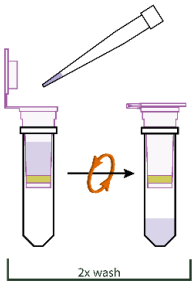
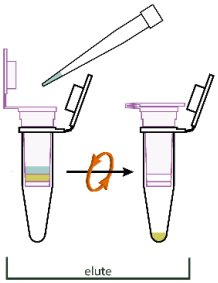
- If the extraction tubes used are coated with carrier nucleic acids and internal control RNA, it is not necessary to use the CARRIER. The minimum amount of CARRIER should be optimized for each PCR method. Excessive CARRIER can inhibit PCR.
- The use of an internal control RNA as well as positive and negative controls to monitor the purification, amplification and detection processes is highly recommended. Control RNA can be added when preparing the CARRIER–Buffer LYSIS LR mix.
- Perform all centrifugation steps at room temperature.
- Before elution, the necessary volume of Water, RNase-free (for ELUTION), must be warmed to 70°C.
- Final eluates contain both viral RNA and CARRIER, and, therefore, the photometric or fluorometric quantification of nucleic acids is not relevant. qPCR is recommended to quantify the purified RNA.
- The sensitivity of virus detection is highly dependent on the procedure used (standard PCR or commercial detection kits).
- Mark all vials and filters to avoid confusion when purifying multiple samples.

GUIDELINES FOR PREVENTION OF RNA DEGRADATION

Special care should be taken to minimize contamination with RNases, as RNA is extremely sensitive to degradation.

- Always wear gloves, and change them frequently.
- Keep all tubes closed when possible.
- Keep samples and 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. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware.
  - Autoclaving alone will not inactivate many RNases completely. The glassware should be immersed in 0.1% diethylpyrocarbonate (DEPC) solution for 12 h at 37°C before autoclaving or heating 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 must be prepared with DEPC-treated RNase-free double-distilled water.
- 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.

SHORT PROTOCOL

STEPS	SCHEME
• Homogenize and lyse the sample material. • Centrifuge to pellet debris.	
• Add Buffer BINDING BR and transfer to a Mini Filter (bordeaux). • Centrifuge.	
• Add Buffer WASH A and centrifuge. • Add Buffer WASH B and centrifuge. • Centrifuge once more to remove residual ethanol.	
• Add Water, RNase-free (for ELUTION), incubate and centrifuge. • RNA in the Elution Tube is ready for use.	

PROTOCOL FOR ISOLATION OF VIRAL RNA FROM SERUM, PLASMA, CELL-FREE BODY FLUIDS AND CELL CULTURE SUPERNATANTS UP TO 150 µL

PROCEDURE	NOTES
• Transfer 450 µl fresh CARRIER–Buffer LYSIS LR mix to an empty 1.5 ml reaction tube. • Add 150 µl sample (serum, plasma, cell-free body fluids or cell culture supernatants). • Add 20 µl Proteinase K. • Mix by pulse vortexing for 10 s. • Incubate at room temperature or 37°C until the sample is completely lysed (approximately 10–30 min). • Centrifuge briefly to collect the liquid at the bottom of the tube.	• Before use, prepare the CARRIER–Buffer LYSIS LR mix as described above. • Use a shaking platform (thermomixer, water bath or other rocking platform) to ensure continuous shaking during lysis. Alternatively, vortex the sample 3–4 times during the incubation. • The solution becomes clear when the lysis is complete. Incubation time depends on sample and temperature. Incomplete lysis can decrease RNA yield.
• Add 600 µl Buffer BINDING BR to the lysate, and mix by vortexing or by pipetting up and down several times.	Mix the viscous buffer carefully. Option: Add internal control at this step.
• Apply 650 µl sample to a Mini Filter (bordeaux) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Apply the remaining sample volume to the Mini Filter placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	
• Add 500 µl Buffer WASH A to the Mini Filter placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH A as described above.
• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH B as described above.
• Place the Mini Filter into a new Collection Tube. • Centrifugation again for 3 min to remove residual ethanol. • Discard the Collection Tube.	
• Place the Mini Filter into an Elution Tube. • Add 60–100 µl warm Water, RNase-free (for ELUTION), to the center of the Mini Filter. • Incubate at room temperature for 2 min. • Centrifuge at 8,000 × *g* (10,000 rpm) for 1 min. • Discard the Mini Filter RNA.	• Before use, prewarm the Water, RNase-free (for ELUTION), to 70°C. • To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION).
• Purified RNA in the Elution Tube can be used immediately.	• Store the RNA at 4°C (short-term) or –80°C (long-term).

PROTOCOL FOR ISOLATION OF VIRAL RNA FROM SERUM, PLASMA, CELL-FREE BODY FLUIDS AND CELL CULTURE SUPERNATANTS UP TO 300 µL

PROCEDURE	NOTES
• Transfer 400 µl fresh CARRIER–Buffer LYSIS LR mix to an empty 1.5 ml reaction tube. • Add 300 µl sample (serum, plasma, cell-free body fluids or cell culture supernatants). • Add 20 µl Proteinase K. • Mix by pulse vortexing for 10 s. • Incubate at room temperature or 37°C until the sample is completely lysed (approximately 10–30 min). • Centrifuge briefly to collect the liquid at the bottom of the tube.	• Before use, prepare the CARRIER–Buffer LYSIS LR mix as described above. • Use a shaking platform (thermomixer, water bath or other rocking platform) to ensure continuous shaking during lysis. Alternatively, vortex the sample 3–4 times during the incubation. • The solution becomes clear when the lysis is complete. Incubation time depends on sample and temperature. Incomplete lysis can decrease RNA yield.
• Add 700 µl Buffer BINDING BR to the lysate, and mix by vortexing or by pipetting up and down several times.	Mix the viscous buffer carefully. Option: Add internal control at this step.
• Apply 650 µl sample to a Mini Filter (bordeaux) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Apply the remaining sample volume to the Mini Filter placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	
• Add 500 µl Buffer WASH A to the Mini Filter placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH A as described above.
• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH B as described above.
• Place the Mini Filter into a new Collection Tube. • Centrifugation again for 3 min to remove residual ethanol. • Discard the Collection Tube.	
• Place the Mini Filter into an Elution Tube. • Add 60–100 µl warm Water, RNase-free (for ELUTION), to the center of the Mini Filter. • Incubate at room temperature for 2 min. • Centrifuge at 8,000 × *g* (10,000 rpm) for 1 min. • Discard the Mini Filter RNA.	• Before use, prewarm the Water, RNase-free (for ELUTION), to 70°C. • To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION).
• Purified RNA in the Elution Tube can be used immediately.	• Store the RNA at 4°C (short-term) or –80°C (long-term).

PROTOCOL FOR ISOLATION OF VIRAL RNA FROM TISSUE SAMPLES AND BIOPSIES

PROCEDURE	NOTES
• Cut up to 20 mg tissue sample or biopsy into small pieces. • Add 9 volumes PBS buffer or Water, RNase-free, to make a 10% (w/v) tissue suspension. • Homogenized the sample using a commercial homogenization tool (bead-based or other).	• Insufficient homogenization can decrease RNA yields. • Avoid repeated freezing and thawing of tissue samples.
• Centrifuge at maximum speed for 2 min to pellet debris. Use the supernatant in the next step.	
• Transfer 450 µl fresh CARRIER–Buffer LYSIS LR mix to an empty 1.5 ml reaction tube. • Add 150 µl particle-free sample (homogenized tissue). • Add 20 µl Proteinase K. • Mix by pulse vortexing for 10 s. • Incubate at room temperature or 37°C until the sample is completely lysed (approximately 10–30 min). • Centrifuge briefly to collect the liquid at the bottom of the tube.	• Before use, prepare the CARRIER–Buffer LYSIS LR mix as described above. • Use a shaking platform (thermomixer, water bath or other rocking platform) to ensure continuous shaking during lysis. Alternatively, vortex the sample 3–4 times during the incubation. • The solution becomes clear when the lysis is complete. Incubation time depends on sample and temperature. Incomplete lysis can decrease RNA yield.
• Add 450 µl Buffer BINDING BR to the lysate, and mix by vortexing or by pipetting up and down several times.	Mix the viscous buffer carefully. Option: Add internal control at this step.
• Apply 650 µl sample to a Mini Filter (bordeaux) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Apply the remaining sample volume to the Mini Filter placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	
• Add 500 µl Buffer WASH A to the Mini Filter placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH A as described above.
• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH B as described above.
• Place the Mini Filter into a new Collection Tube. • Centrifugation again for 3 min to remove residual ethanol. • Discard the Collection Tube.	

• Place the Mini Filter into an Elution Tube. • Add 60–100 µl warm Water, RNase-free (for ELUTION), to the center of the Mini Filter. • Incubate at room temperature for 2 min. • Centrifuge at 8,000 × *g* (10,000 rpm) for 1 min. • Discard the Mini Filter RNA.	• Before use, prewarm the Water, RNase-free (for ELUTION), to 70°C. • To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION).
• Purified RNA in the Elution Tube can be used immediately.	• Store the RNA at 4°C (short-term) or −80°C (long-term).

PROTOCOL FOR ISOLATION OF VIRAL RNA FROM SWABS

PROCEDURE	NOTES
• Transfer 0.9% NaCl to a 1.5 ml reaction tube. • Submerge the swab in the liquid. If the shaft of the swab interferes with closing the tube, it can be cut off. • Incubate at room temperature for 15 min.	• Before use, prepare the 0.9% NaCl as described above. • Insufficient lysis can decrease RNA yield.
• After incubation, mix well. Shake the liquid from the swab into the tube, and squeeze the swab on the wall of the tube to transfer as much liquid as possible to the tube. • Discard the swab.	
• Transfer 450 µl fresh CARRIER–Buffer LYSIS LR mix to an empty 1.5 ml reaction tube. • Add 150 µl particle-free sample (liquid from swab). • Add 20 µl Proteinase K. • Mix by pulse vortexing for 10 s. • Incubate at room temperature or 37°C until the sample is completely lysed (approximately 10–30 min). • Centrifuge briefly to collect the liquid at the bottom of the tube.	• Before use, prepare the CARRIER–Buffer LYSIS LR mix as described above. • Use a shaking platform (thermomixer, water bath or other rocking platform) to ensure continuous shaking during lysis. Alternatively, vortex the sample 3–4 times during the incubation. • The solution becomes clear when the lysis is complete. Incubation time depends on sample and temperature. Incomplete lysis can decrease RNA yield.
• Add 450 µl Buffer BINDING BR to the lysate, and mix by vortexing or by pipetting up and down several times.	Mix the viscous buffer carefully. Option: Add internal control at this step.
• Apply 650 µl sample to a Mini Filter (bordeaux) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.

- Apply the remaining sample volume to the Mini Filter placed in a new Collection Tube.
- Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min.
- Discard the Collection Tube with the filtrate.

- Add 500 µl Buffer WASH A to the Mini Filter placed in a new Collection Tube.
- Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min.
- Discard the Collection Tube with the filtrate.

- Before use, prepare Buffer WASH A as described above.

- Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube.
- Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min.
- Discard the Collection Tube with the filtrate.

- Before use, prepare Buffer WASH B as described above.

- Place the Mini Filter into a new Collection Tube.
- Centrifugation again for 3 min to remove residual ethanol.
- Discard the Collection Tube.

- Place the Mini Filter into an Elution Tube.
- Add 60–100 µl warm Water, RNase-free (for ELUTION), to the center of the Mini Filter.
- Incubate at room temperature for 2 min.
- Centrifuge at 6000 × *g* (8,000 rpm) for 1 min.
- Discard the Mini Filter RNA.

- Before use, prewarm the Water, RNase-free (for ELUTION), to 70°C.
- To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION).

- Purified RNA in the Elution Tube can be used immediately.

- Store the RNA at 4°C (short-term) or −80°C (long-term).

TROUBLESHOOTING

PROBLEM	SOLUTION
CLOGGED MINI FILTER	
Excessive starting material, insufficient lysis	Follow recommendations for the maximum amount of starting material. Perform lysis until the solution becomes clear and viscosity decreases.
LOW YIELD	
Excessive starting material, insufficient lysis	Follow recommendations for the maximum amount of starting material. Perform lysis until the solution becomes clear and viscosity decreases. Higher temperatures are more effective.
Incomplete elution	Prolong the elution time up to 5 min. Repeat elution. Use the recommended volume of Water, RNase-Free.
Incorrect binding	Ensure the sample and Buffer BINDING BR are mixed to homogeneity.
LOW RNA CONCENTRATION	
Excessive elution volume	Prewarm the Water, RNase-free (for ELUTION), as described. Do not exceed the recommended volume of water. Perform two elution steps with half of the total elution volume. The first eluate normally exhibits a higher RNA concentration than the second eluate.
No CARRIER used	Use the CARRIER as described to increase yield and nucleic acid concentration.
DEGRADED RNA	
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, tubes, etc.	Use sterile, RNase-free filter tips. Before every RNA preparation, clean the pipette, devices and working place. Always wear gloves.
RNA DOES NOT PERFORM WELL IN OTHER APPLICATIONS (RT-PCR)	
Ethanol carryover	Increase centrifugation time for removing ethanol.
Salt carryover during elution	Ensure that Buffer WASH A and Buffer WASH B are at room temperature. If a buffer contains precipitate, dissolve the precipitate by warming carefully.

SAFETY PRECAUTIONS

- This kit is made for single use only!
- Don't eat or drink components of the kit!
- The kit shall only be handled by educated personal in a laboratory environment!
- Wear gloves while handling these reagents, and avoid skin contact! In case of contact, flush with water immediately!
- Handle and discard waste according to local safety regulations!
- Do not add bleach or acidic components to the waste after sample preparation!
- Buffers LYSIS LR, BINDING BR and WASH A contain guanidine isothiocyanate, which is harmful to health. Contact with acids liberates very toxic gas!

CERTIFICATE OF ANALYSIS

The components of the kit were tested for viral RNA purification from liquid samples and subsequent analysis of purified RNA in qPCR. The kit was tested for the following viruses: human immunodeficiency, hepatitis C, influenza A, influenza B, influenza A/H1N1, Rift Valley fever, dengue virus, yellow fever, foot-and-mouth disease and bovine viral diarrhea.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter: http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

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Certified Quality-System mdc ISO 13485 ISO 9001

Legal Disclaimer and Product Use Limitation

Purchase of product does not include a license to perform any patented applications; therefore it is the sole responsibility of users to determine whether they may be required to engage a license agreement depending upon the particular application in which the product is used. This product was developed, manufactured, and sold for in vitro use only. It is not suitable for administration to humans or animals.
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valid from 26.07.2021