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PuroSPIN™ Genomic DNA Purification Kit

- *Genomic DNA Purification from Gram-Negative/Positive Bacteria*
- *Genomic DNA Purification from Yeast*
- *Genomic DNA Purification from Mammalian Cells*
- *Genomic DNA Purification from Mammalian Blood*
- *Genomic DNA Purification from Animal Tissue*

#NK061-50, #NK061-250

Product Manual

Version 2.2

For research use only. Not for use in diagnostic procedures.

Kit Contents

Component	50 Preps (#NK061-50)	250 Preps (#NK061-250)
Lysis Buffer LB-GD1	30 mL	125 mL
Lysis Buffer LB-GD2	30 mL	125 mL
Wash Buffer WB-GD (*after Ethanol addition*)	*120 mL*	*2 x 225 mL*
Elution Buffer EB	15 mL	50 mL
Water, Nuclease-free	15 mL	50 mL
Proteinase K (20 mg/mL), Nuclease-free	2 x 1 mL	5.5 mL
RNase A (20 mg/mL), DNase-free	2 x 1 mL	5.5 mL
PuroSPIN™ MINI Spin Columns	50	250
Collection Tubes (2 mL)	50	250

Storage

Proteinase K and **RNase A** need to be stored at **≤ -20°C**. All other components can be stored at **room temperature** for at least 1 year.

Additional materials and equipment required

- 96-100% Ethanol
- 1.5 mL microcentrifuge tubes
- Vortex (if not available, can be replaced with vigorous pipetting)
- Appropriate personal protective equipment (gloves, lab coat)

For DNA purification from Gram-positive bacteria:

- **Bacterial Lysis Buffer** (20 mM Tris-HCl, pH 8, 2 mM EDTA, 1.5% Triton X-100). Add lysozyme at a final concentration of 20 mg/mL immediately before use.

For DNA purification from yeast:

- Bead-beater homogenizer (with 0.5-1 mm glass or zirconia beads); or **Yeast Lysis Buffer** (1 M Sorbitol, 0.1M EDTA, pH 7.4), lyticase / zymolase enzyme.

For DNA purification from mammalian cells:

- PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)

For DNA purification from animal tissue (optional):

- Rotor-stator homogenizer, or bead-beater homogenizer (with 1-2 mm zirconia or steel beads), or mortar and pestle with liquid nitrogen.

Kit Description

PuroSPIN™ Genomic DNA Purification Kit is designed to purify genomic DNA from different biological samples such as bacteria, yeast, mammalian cells, blood and animal tissue. It greatly simplifies the purification process by incorporating a convenient easy-to-use extraction technology based on nucleic acid binding silica spin-columns. The DNA is first released from cells or tissue by the treatment with the lysis buffers LB-GD1, LB-GD2 and Proteinase K. DNA is then loaded onto silica-based spin columns and washed to remove protein, salt and other contaminants. Finally, purified DNA is eluted from the column into a clean microcentrifuge tube in **Elution Buffer EB**, or **nuclease-free water**. High quality DNA extracted with the **PuroSPIN™ Genomic DNA Purification Kit** can be used for a range of downstream applications, such as PCR, Southern blotting, or genomic sequencing.

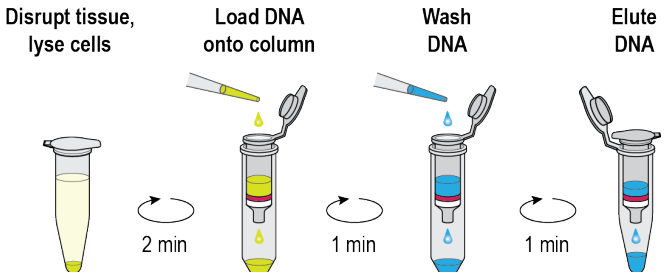
Important Notes

BEFORE USE:

- Add 96-100% Ethanol to the **Wash Buffer WB-GD**:
  - **#NK061-50** (50 preps): add **90 mL** of Ethanol
  - **#NK061-250** (250 preps): add **170 mL** of Ethanol

- **Lysis Buffers LB-GD1 and LB-GD2** contain ***guanidine hydrochloride***. It is a known irritant that is harmful if inhaled, swallowed, or contacted with skin. Wear appropriate protective equipment when working with this reagent.
- If precipitate is observed in the **Lysis Buffers LB-GD1 and LB-GD2**, re-dissolve it by warming the solution to 37°C. Cool the solution back to room temperature before use.

Experimental Workflow



Protocol A. Purification of Genomic DNA from Gram-Negative Bacteria

Step	Procedure
1	Transfer 1.5 mL of Gram-negative bacterial culture (up to 10^9 CFU/mL) into a clean 1.5 mL microcentrifuge tube. Pellet the bacteria by spinning the tube at 12,000g for 2 min. Carefully remove and discard as much of the supernatant as possible without disturbing the bacterial pellet.
2	To each tube add 200 µL of the **Lysis Buffer LB-GD1**. Pipette up and down until homogenous suspension of cells is achieved.
3	Add 20 µL of **Proteinase K** to the solution. Incubate the cell suspension for 20 min at 55ºC.
4	Add 20 µL of 20 mg/mL of **RNase A** to the solution. Incubate for 10 min at room temperature.
5	Add 200 µL of **Lysis buffer LB-GD2**. Invert the tube 4-6 times to mix.
6	Add 200 µL of 100% **Ethanol**. Invert the tube a few times to mix.
7	Transfer up to 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through. *NOTE 1: If the total solution exceeds 700 µL, repeat this step to load the remaining solution onto the column.* *NOTE 2: If the liquid is still observed in the column above the filter following the 2 min centrifugation step, repeat the centrifugation until all the liquid has passed through the column.*
8	Add 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
9	Add another 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
10	Centrifuge at 12,000g for 1 min to dry out the membrane.
11	Transfer the **PuroSPIN™ MINI Spin Column** into a new DNase-free 1.5 mL microcentrifuge tube.
12	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the DNA. Keep the flow-through that contains the product and discard the column.

Protocol B. Purification of Genomic DNA from Gram-Positive Bacteria

If performing Gram-positive bacterial cell disruption by the enzymatic lysis method (Step 2a), *within 30 min of starting the DNA purification* add **Lysozyme** to the **Bacterial Lysis Buffer** (20 mM Tris-HCl, pH 8, 2 mM EDTA, 1.5% Triton X-100) to a final concentration of 20 mg/mL. Vortex well to dissolve.

NOTE: We highly recommend the use of bead-beater homogenizer disruption method for the purification of DNA from Gram-positive bacteria (Step 2b), as it disrupts bacterial cells much better than the enzymatic digestion method (Step 2a), and therefore results in significantly higher genomic DNA recovery.

Step	Procedure
1	Transfer 1.5 mL of Gram-positive bacterial culture (up to 10^9 CFU/mL) into a clean 1.5 mL microcentrifuge tube. Pellet the bacteria by spinning the tube at 12,000g for 2 min. Use a pipette to carefully remove and discard as much of the supernatant as possible without disturbing the bacterial pellet.
2	Disrupt the bacterial cells by one of the following methods: a) Enzymatic digestion ▪ Re-suspend the cells pelleted in Step 1 in 200 µL of **Bacterial Lysis Buffer** (with lysozyme added). ▪ Incubate for 30 min at 37ºC. ▪ Add 200 µL of the **Lysis buffer LB-GD1**. Pipette the solution up and down until homogenous suspension of cells is achieved b) Bead-beater homogenizer ▪ Re-suspend the cells pelleted in Step 1 in 400 µL of the **Lysis Buffer LB-GD1**. Vortex vigorously or pipette up and down until the pellet is resuspended. ▪ Transfer the sample into a new DNase-free 2 mL screw cap tube containing ~300 µL of 0.1 mm glass or zirconia beads (ensure the tube is compatible with the bead-beater apparatus). ▪ Disrupt the sample for 2-3 min using a bead-beater homogenizer (please refer to manufacturer's manual for specific instructions). ▪ Centrifuge the samples at 100g for 1 min to pellet the beads. Transfer the supernatant into a new DNase-free 1.5 mL microcentrifuge tube.
3	Add 20 µL of 20 mg/mL **Proteinase K** to the solution. Incubate the cell suspension for 20 min at 55ºC.
4	Add 20 µL of 20 mg/mL of **RNase A** to the solution. Incubate for 10 min at room temperature.

5	Add 200 µL of the **Lysis Buffer LB-GD2**. Mix by inverting the tube 4-6 times.
6	Add 300 µL of 100% **Ethanol**. Invert the tube a few times to mix.
7	Transfer up to 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through. *NOTE 1: If the total solution exceeds 700 µL, repeat this step to load the remaining solution onto the column.* *NOTE 2: If the liquid is still observed in the column above the filter following the 2 min centrifugation step, repeat the centrifugation until all the liquid has passed through the column.*
8	Add 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
9	Add another 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
10	Centrifuge at 12,000g for 1 min to dry out the membrane.
11	Transfer the **PuroSPIN™ MINI Spin Column** into a new DNase-free 1.5 mL microcentrifuge tube.
12	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the DNA.

Protocol C. Purification of Genomic DNA from Yeast

If performing yeast cell disruption by the enzymatic lysis method (Step 2a), prepare **Yeast Lysis Buffer** containing 1 M sorbitol, 0.1 M EDTA, pH 7.4. *Immediately before use*, add β-mercaptoethanol to a final concentration of 0.1% and lyticase/zymolase enzyme in the amount of 50 U for each 10^7 yeast cells.

Step	Procedure
1	Transfer 1.5 mL of yeast culture (up to 10^8 CFU/mL) into a clean 1.5 mL microcentrifuge tube. Pellet the yeast by spinning the tube at 3,000g for 2 min. Use a pipette to carefully remove and discard as much of the supernatant as possible without disturbing the bacterial pellet.
2	Disrupt the yeast cells by one of the following methods: a) Enzymatic digestion ▪ Resuspend the pellet in 500 µL of the **Yeast Lysis Buffer** supplemented with β-mercaptoethanol and lyticase / zymolase enzymes. Incubate the solution for 1 hour at 37ºC with gentle shaking to generate spheroplasts. Do not vortex the solution since this might disrupt the spheroplasts. ▪ Centrifuge the solution at 300g for 5 min to pellet the spheroplasts. Carefully remove and discard the supernatant. ▪ Re-suspend the pelleted cells in 200 µL of the **Lysis Buffer LB-GD1**. Pipette up and down until homogenous suspension of cells is achieved. ▪ Add 20 µL of 20 mg/mL **Proteinase K** to the solution. Incubate the cell suspension at 55ºC for 40 min or until the cells are completely digested. b) Bead-beater homogenizer ▪ Re-suspend the cells pelleted in Step 1 in 300 µL of the **Lysis Buffer LB-GD1**. Vortex vigorously or pipette up and down until the pellet is resuspended. ▪ Transfer the sample into a new DNase-free 2 mL screw cap tube containing ~300 µL of 0.5-1 mm glass or zirconia beads (ensure the tube is compatible with the bead-beater apparatus). ▪ Disrupt the sample for 2-3 min using the bead-beater homogenizer (please refer to manufacturer's manual for specific instructions). ▪ Centrifuge the samples at 100g for 1 min to pellet the beads. Transfer the supernatant into a new DNase-free 1.5 mL microcentrifuge tube. ▪ Add 20 µL of 20 mg/mL **Proteinase K** to the solution. Incubate the cell suspension at 55ºC for 10 min.
3	Add 20 µL of 20 mg/mL **RNase A** to the solution from Step 2. Incubate for 10 min at room temperature.

4	Add 200 µL of the **Lysis Buffer LB-GD2**. Mix by inverting the tube 4-6 times.
5	Add 200 µL of 100% **Ethanol**. Invert the tube a few times to mix.
6	Transfer up to 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through. *NOTE 1: If the total solution exceeds 700 µL, repeat this step to load the remaining solution onto the column.* *NOTE 2: If the liquid is still observed in the column above the filter following the 2 min centrifugation step, repeat the centrifugation until all the liquid has passed through the column.*
7	Add 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
8	Add another 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
9	Centrifuge at 12,000g for 1 min to dry out the membrane.
10	Transfer the **PuroSPIN™ MINI Spin Column** into a new DNase-free 1.5 mL microcentrifuge tube.
11	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the DNA. Keep the flow-through that contains the product and discard the column.

Protocol D. Purification of Genomic DNA from Mammalian Cells

Step	Procedure
1	a) Suspension cells Centrifuge up to 10^7 cells in an appropriate centrifuge tube at 300g for 5 min. Discard the supernatant. Wash the cell pellet with 5 mL of PBS to remove any residual growth medium. Repeat the centrifugation. Use a pipette to carefully remove and discard as much of the supernatant as possible without disturbing the cell pellet. b) Adherent cells Remove growth medium from the plate with up to $5x10^6$ adherent cells. Wash the cells with 5 mL of PBS to remove residual growth medium, discard PBS. Detach the cells by scraping in an appropriate volume of PBS or trypsinization. If the volume of cell suspension is above 1.5 mL, centrifuge cells at 300g for 5 min and resuspend in 1 mL of PBS. Transfer the cell suspension into a new DNase-free 1.5 mL microcentrifuge tube and pellet the cells by centrifugation at 300g for 5 min. Use a pipette to carefully remove and discard as much of the supernatant as possible without disturbing the cell pellet.
2	To each tube add 200 µL of the **Lysis Buffer LB-GD1**. Pipette up and down until homogenous suspension of cells is achieved.
3	Add 20 µL of 20 mg/mL **Proteinase K** to the solution. Incubate the cell suspension for 20 min at 55ºC.
4	Add 20 µL of 20 mg/mL of **RNase A** to the solution. Incubate for 10 min at room temperature.
5	Add 200 µL of the **Lysis Buffer LB-GD2**. Mix by inverting the tube 4-6 times.
6	Add 200 µL of 100% **Ethanol**. Invert the tube a few times to mix.
7	Transfer up to 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through. *NOTE 1: If the total solution exceeds 700 µL, repeat this step to load the remaining solution onto the column.* *NOTE 2: If the liquid is still observed in the column above the filter following the 2 min centrifugation step, repeat the centrifugation until all the liquid has passed through the column.*
8	Add 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.

9	Add another 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
10	Centrifuge at 12,000g for 1 min to dry out the membrane.
11	Transfer the **PuroSPIN™ MINI Spin Column** into a new DNase-free 1.5 mL microcentrifuge tube.
12	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the DNA. Keep the flow-through that contains the product and discard the column.

Protocol E. Purification of Genomic DNA from Whole Mammalian Blood

Step	Procedure
1	Transfer 200 µL of Whole Blood into a clean 1.5 mL microcentrifuge tube. Add 2 volumes (400 µL) of the **Lysis Buffer LB-GD2**. Pipette up and down until homogenous suspension is achieved.
2	Add 20 µL of 20 mg/mL **Proteinase K** to the solution and incubate for 10 min at 55ºC.
3	Add 1 volume (200 µL) of 100% **Ethanol**. Invert a few times to mix.
4	Transfer up to 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through. *NOTE 1: If the total solution exceeds 700 µL, repeat this step to load the remaining solution onto the column.* *NOTE 2: If the liquid is still observed in the column above the filter following the 2 min centrifugation step, repeat the centrifugation until all the liquid has passed through the column.*
5	Add 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
9	Add another 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
10	Centrifuge at 12,000g for 1 min to dry out the membrane.
11	Transfer the **PuroSPIN™ MINI Spin Column** into a new DNase-free 1.5 mL microcentrifuge tube.
12	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the DNA. Keep the flow-through that contains the product and discard the column.

Protocol F. Purification of Genomic DNA from Animal Tissue

Step	Procedure
1	Collect up to 30 mg of fresh or frozen tissue. For rodent tail, collect a tail fragment of up to 6 mm in length. *NOTE: Cutting the tissue into small pieces (~1-2 mm in cross-section) will speed up homogenization / digestion.*
2	Homogenize the tissue using one of the following methods: a) Rotor-stator homogenizer ▪ Transfer the tissue sample into an appropriate container. Add 400 µL of **Lysis Buffer LB-GD1** into the tube. ▪ Homogenize the sample for 20-50 seconds using standard rotor-stator homogenizer (please refer to the apparatus manufacturer's manual for specific instructions). b) Bead-beater homogenizer ▪ Transfer the samples into a new 2 mL DNase-free screw cap tube (ensure the tube is compatible with the bead-beater apparatus). ▪ Add 400 µL of **Lysis Buffer LB-GD1** into the tube. ▪ Add 8-10 of 2-3 mm steel beads to the sample tube using a spatula. Disrupt the tissue for 3-5 min using a bead-beater homogenizer (please refer to bead-beater apparatus manufacturer's manual for specific instructions). c) Mortar and pestle homogenization ▪ Freeze the tissue by immersing the tube containing it into liquid nitrogen. Grind the tissue thoroughly using mortar and pestle. ▪ Add 400 µL of **Lysis Buffer LB-GD1** to the tissue. ▪ Disrupt the tissue further by passing it 5-6 times through a 20-gauge needle using DNase-free syringe. d) Direct digestion (no homogenization) ▪ Add 400 µL of **Lysis Buffer LB-GD1** to the tissue. *NOTE 1: Tissues can be digested directly without prior homogenization (Option d). However, longer Proteinase K digestion times are required in this case.* *NOTE 2: If homogenizing elastic or hard animal tissue (skin, cartilage, bone, tail, nails) using bead-beater (Option b), use a 4 mL tube with a single 6-7mm steel ball. Increase the volumes of Lysis Buffer LB-GD1, Lysis Buffer LB-GD2, and 100% Ethanol to 1 mL.*
3	Add 20 µL of 20 mg/mL **Proteinase K** to the solution and incubate at 55ºC until the tissue is completely lysed (typically ~1-2 hours).

	NOTE: If the tissue was not homogenized (Step 2d), the digestion time should be extended to ~6-8 hours.
4	Add 20 µL of 20 mg/mL of **RNase A** to the solution. Incubate for 10 min at room temperature.
5	Centrifuge the homogenized sample tube at 12,000g for 3 min to pellet any undigested components (and the beads if Step 2b was followed). Transfer the supernatant into a new DNase-free 1.5 mL microcentrifuge tube.
6	Add 400 µL of the **Lysis Buffer LB-GD2**. Mix by inverting the tube 4-6 times.
7	Add 1 volume (400 µL) of 100% **Ethanol**. Invert a few times to mix.
8	Transfer up to 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through. Repeat this step until all of the solution has been loaded onto the column.
9	Add 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
10	Add another 700 µL of **Wash Buffer WB-GD** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
11	Centrifuge at 12,000g for 1 min to dry out the membrane.
12	Transfer the **PuroSPIN™ MINI Spin Column** into a new DNase-free 1.5 mL microcentrifuge tube.
13	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the DNA. Keep the flow-through that contains the product and discard the column.

Troubleshooting

Problem	Possible causes and solutions
Column clogging	**Too much starting material was used.** Do not exceed the amount of material indicated in the protocol. **Tissue digestion was not complete.** Extend Proteinase K digestion at 55ºC until the sample is completely lysed and no particulates remain. Homogenize the sample prior to Proteinase K digestion.
Low DNA yield	**Ethanol was not added to Wash Buffer WB-GD2** Add the indicated amount of 96-100% to the buffer before use. **Too much starting material was used causing column to clog.** Reduce the amount of material used for the lysate preparation. **Ethanol was not added to the lysate.** Make sure the indicated amount of ethanol is added to the sample lysate before loading it onto the column. **Starting material was not completely digested.** Extend Proteinase K digestion at 55ºC until the sample is completely lysed and no particulates remain. Homogenize the sample prior to Proteinase K digestion.
Degraded DNA	**DNase contamination.** Use DNase-free tips and tubes. Clean work surfaces and non-disposable items with DNase removal solution. Wear gloves. **Sample was not properly stored.** Bacteria, mammalian cells and animal tissues should be stored at temperatures ≤ -20°C. Whole blood can be stored at 4°C for 1-2 days, but should be frozen at ≤ -20°C for longer storage. **Sample was repeatedly frozen and thawed.** Avoid multiple freeze/thaw cycles, since they can damage DNA. Extract genomic DNA from fresh samples whenever possible.
RNA contam-ination	**RNase A digestion was not performed.** Follow the instructions to carry out the RNase A digestion step as described in the manual.
Inhibition of downstream enzymatic reactions	**Purified Genomic DNA contains residual salts.** Wash the column 2X with the indicated amount of Wash Buffer WB-GD2. If you have scaled up the sample size and/or volumes of lysis buffers, add additional washing steps using Wash Buffer WB-GD2. **Purified DNA contains residual Ethanol.** After washing the column with Wash Buffer WB-GD2, dry the column by centrifuging it at 12,000g for 1 min as indicated in the protocol.

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Lysis Buffer LB-GD2		Contains ***guanidine hydrochloride*** • Harmful if swallowed. • Irritating to eyes and skin. • Do not breathe vapors and fumes • Wear suitable protective clothing and gloves. • In case of contact with eyes immediately wash with plenty of water and seek medical advice. • If swallowed, seek medical advice and contact poison control center.

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. It has not tested for use in diagnostics or for drug development. It is not suitable for administration to humans or animals.

Full material safety data sheet document is available at www.lunanano.com.

For further information about this and other products from Luna Nanotech please refer to our website at www.lunanano.com.

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