SmartLid™
Blood DNA/RNA Extraction Starter Kit

Research Use Only. Not for use in diagnostic procedures.

Catalogue Number: 100373 **Quantity: 50 Extractions**

Definition of symbols used	
	Instructions for use
REF	Reference number or Catalogue Number
	Manufacturer
	Storage temperature range
LOT	LOT Number
	Expiry Date
	Corrosive
	Harmful
	Serious health hazard

Resources and Training Videos

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Contents

1. Introduction

Product Description

The SmartLid Blood DNA/RNA Extraction Kit has been designed for the rapid extraction of DNA and RNA from whole blood. Uniquely, the kit is capable of lysing and purifying nucleic acids from viruses, gram-negative bacteria, gram-positive bacteria, and even certain parasites, with all required buffers and enzymes included.

Purified extractions are suitable for a variety of downstream molecular applications, such as amplification reactions (RT-PCR, RT-LAMP, etc.) and further analytical procedures.

The SmartLid Blood DNA/RNA Extraction Kit leverages a novel (patent pending) sample preparation method for nucleic acid extraction, centring around a proprietary magnetic lid, called SmartLid, to transfer nucleic acids through three simple steps: Lysis, Wash, and Elution. SmartLid requires no centrifugation and enables ultra-fast, user-friendly, and economical nucleic acid extractions. The procedure is based on magnetic separation and utilizes the fastest collecting superparamagnetic beads on the market (TurboBeads™).

Advantages

Key Features	
High yield	Ultra-pure DNA/RNA for sensitive downstream applications in molecular biology
Rapid	From sample to eluted DNA/RNA in as little as 20 minutes, with fastest Magnetic Beads on the market (TurboBeads™)
Easy to use	Simple process that does not require any centrifugation and minimizes pipetting
Broad compatibility	Efficiently lyse and extract nucleic acids from viruses, bacteria (both gram-negative and positive), and certain parasites
Enzymes included	Critical digestion enzymes included to conveniently lyse all the above, including gram-positive bacteria
Accessories	SmartLid Rack and SmartLid Vortex Tool enable simultaneous processing of up to 12 samples
Environment	Designed to minimise plastic waste Carton and paper inserts made with recycled content and are fully recyclable Magnetic keys are reusable, reducing rare-earth waste

2. Product Specifications

Key Specifications	
Applications	PCR, RT-PCR, qPCR, RT-qPCR, LAMP, RT-LAMP, dPCR, RT-dPCR (and other amplification chemistries)
Technology	Superparamagnetic beads (TurboBeads™)
Main Sample Types	Whole blood (also compatible with plasma and serum)
Microbe Compatibility	Viruses, gram-negative and gram-positive bacteria, certain parasites (e.g. malaria)
Isolated Molecules	DNA, RNA
Sample Input Volume	100 µl (up to 200 µL possible)
Elution Volume	50 µL recommended
A260/A280 Ratio	1.7-2.0 (typical range)
Protocol time	<20 minutes (viruses and gram-negative bacteria), <45 minutes (gram-positive bacteria)
Storage conditions	Room temperature (15-25°C)
Limitations	Not intended for the diagnosis, prevention, or treatment of a disease.

3. Safety precautions

The components of the ProtonDx SmartLid Blood DNA/RNA extraction kit contain hazardous contents. For comprehensive information on these hazards and proper handling instructions, please refer to the Material Safety Data Sheet (MSDS) available at protondx.com. Alternatively, you can request a copy of the MSDS document by emailing info@protondx.com.

The techniques of “good laboratory practice” should be employed when using the kit. If such practices are used, the reagents constitute a very low potential risk to health. Wear protective gloves/protective clothing/eye protection/face protection. IF ON SKIN: Wash with plenty of water. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Take off contaminated clothing and wash it before reuse. Dispose of contents/container in accordance with national regulations. It is important to be aware of the allergic, toxic, or infectious potential of analytical samples. In all cases of doubt, or if experiencing symptoms following exposure to any ingredients, seek medical advice.

Item	GHS symbol	Hazard Statements	Precautionary Statements
Lysis Buffer BL-1		H302 Harmful if swallowed. H315 Causes skin irritation. H319 Causes serious eye irritation. H335 May cause respiratory irritation. H373 - May cause damage to organs through prolonged or repeated exposure.	P202, P233, P260, P261, P264, P270, P271, P280, P301 + P312, P302 + P352, P305 + P351 + P338, P314, P332 + P313, P337 + P313, P362 + P364, P403, P405, P501
Magnetic Beads		H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties.	P260, P261, P280, P302+P352, P342+P311, P201, P202, P273, P501

		H341 Suspected of causing genetic defects. H350 May cause cancer. H360 May damage fertility. Suspected of damaging the unborn child. H412 Harmful to aquatic life with long lasting effects.	
Enzyme BZ-L		H315 Causes skin irritation. H319 Causes serious eye irritation. H335 May cause respiratory irritation H373 - May cause damage to organs through prolonged or repeated exposure.	P260, P261, P280, P305+ P351+ P338
Enzyme Buffer BZ-P		H315 Causes skin irritation. H319 Causes serious eye irritation. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. H335 May cause respiratory irritation.	P261, P264, P271, P280, P284, P302+P352, P304+P340, P312, P332+P313, P337+P313, P342+P311, P362+P364, P403+P233, P405
Wash Buffer BW-1		H302 Harmful if swallowed. H315 Causes skin irritation. H319 Causes serious eye irritation. H373 - May cause damage to organs through prolonged or repeated exposure.	P202, P233, P260, P264, P270, P271, P280, P301 + P312, P302 + P352, P305 + P351 + P338, P314, P332 + P313, P337 + P313, P362 + P364, P403, P405, P501
Wash Buffer BW-2		H373 - May cause damage to organs through prolonged or repeated exposure.	P260
Elution Buffer BE-1		H373 - May cause damage to organs through prolonged or repeated exposure.	P260

Safety Information	
Caution	When working with this kit always use appropriate PPE and avoid any skin contact.
	All chemicals and biological material should be considered potentially hazardous.
	Lysis Binding bottle contains guanidine-thiocyanate. Ensure tubes are sealed prior to disposal, as when combined with bleach, guanidine-thiocyanate can react to produce a highly toxic gas.
	After use, components should be disposed of using appropriate routes, in compliance with local regulations.
	Aerosol-barrier pipette tips are recommended for pipetting the sample elution.
	This kit contains magnets and magnetic materials. Please handle with care to avoid injury and damage to nearby objects. Magnetic fields may interfere with pacemakers and other medical devices. Consult with a healthcare professional before use if you have a medical implant.
Important	Ensure all reaction tubes are not damaged or cracked prior to use. If the bottles are damaged, wear gloves and protective goggles when discarding the bottles.
	Handle and discard liquid waste according to local health and safety guidelines.
	Do not add bleach or acidic components to the waste after sample preparation.

4. Storage Information

The SmartLid Blood DNA/RNA Extraction Kit should be stored dry at room temperature (15–25°C) and is stable for at least 8 months from production under these conditions. If any kit components show signs of leakage, dispose of appropriately and contact customer support.

For longer storage or if ambient temperatures often exceed 25°C, we suggest storing all buffers at 2-8°C. Note, however, this may increase change of precipitation in buffers BL-1 and BW-1. Before every use make sure that all components are at room temperature. If any precipitate is observed within the provided solutions, dissolve by gentle warming. Note: kit components are not suitable for freezing.

5. Kit Contents

Materials Included			
Qty.	**Item**	**Description**	
1.2 mL	Magnetic Beads	Silica coated magnetic beads	
40 mL	Lysis Buffer BL-1	Lysis and binding buffer*	
1.2 mL	Enzyme Buffer BZ-P	Enzymatic digestion buffer	*For all protocols, including viral and gram-negative bacterial extraction.*
12 mL	Enzyme Buffer BZ-L	Enzymatic digestion buffer	*Add Enzyme Powder BZ-L before use. Once combined, use immediately or store frozen.*
264 mg	Enzyme Powder BZ-L	Digestion enzyme	*For difficult to lyse microbes, e.g. gram-positive bacteria.*
12 mL	Wash Buffer BW-1	Concentrated wash buffer 1	*Add EtOH according to label before use.*
9 mL	Wash Buffer BW-2	Concentrated wash buffer 2**	*Add EtOH according to label before use.*
5 mL	Elution Buffer BE-1	Nucleic acid stabilizing elution buffer	
50 pcs	SmartLid	Proprietary transfer lids compatible with provided flip-cap tubes	
200 pcs	Flip-cap tube (2 mL)	Nuclease-free flip-cap tubes (2 mL) compatible with SmartLid	
12 pcs	Green magnetic key	Reusable Magnetic Keys to use in conjunction with SmartLid	

* Contains guanidine hydrochloride, which is an irritant. Not compatible with disinfecting reagents that contain bleach, refer to Section 3.

** Contains Sodium Azide as a preservative.

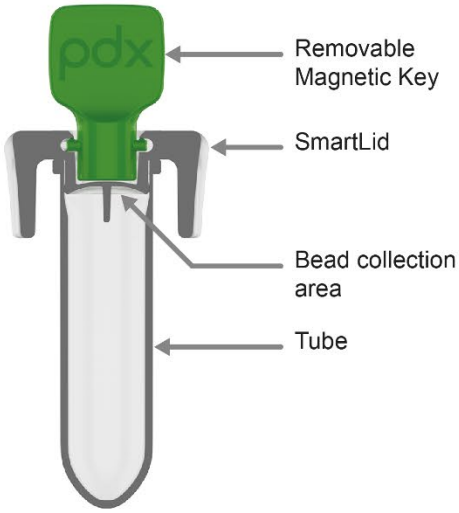
Materials not included (REQUIRED)	
Item	**Purpose**
Molecular biology grade ethanol (>99%)	To add to the Wash Buffer BW-1 and BW-2 before kit use, and during lysis step.

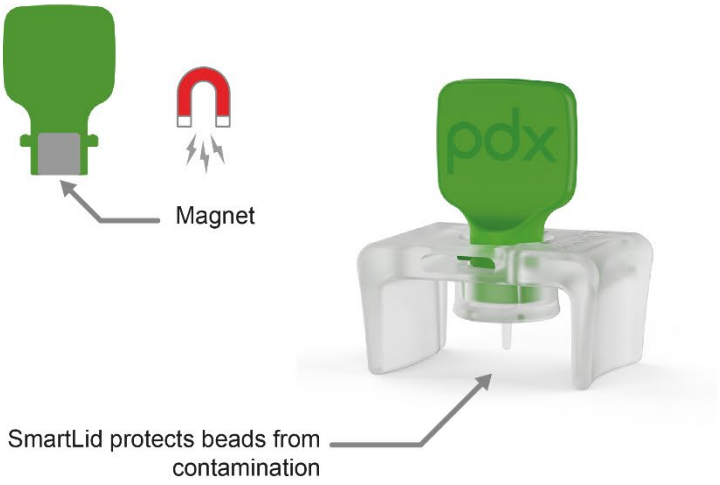
Equipment not included (can be purchased separately)		
Catalogue No.	**Item**	**Description**
100175	SmartLid Rack	Preparation rack to enable easy multi-sample processing
100173	SmartLid Shaker	Multi-tube shaking device to enable high throughput mixing
100444	SmartLid Vortex Tool	Multi-tube vortex mixing device to enable high throughput mixing

6. Visual Guide

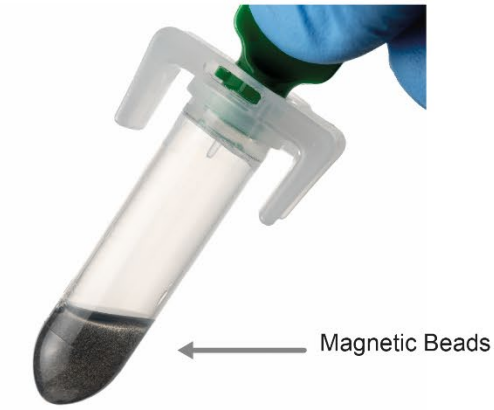
Resources and Training

What is SmartLid?





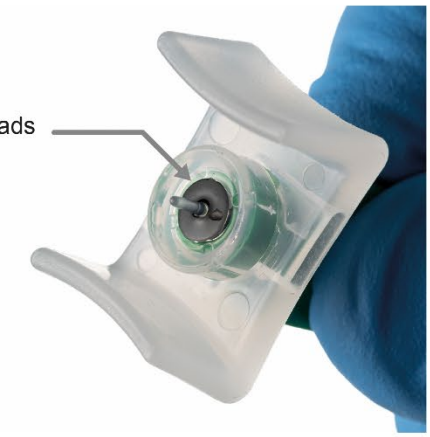
SmartLid in use



1 Magnetic Beads capture nucleic acids for transfer between steps.

2 Inverting the tube with the Magnetic Key inserted collects the Magnetic Beads onto SmartLid.

3 Within seconds the liquid is clear and collection is complete.

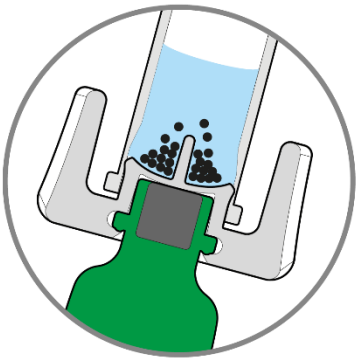


4 Magnetic Beads are safely attached to SmartLid and ready for transfer into the next tube.

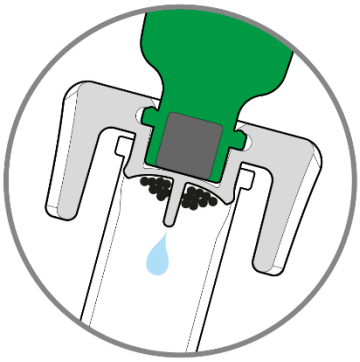
How to transfer Magnetic Beads with SmartLid

SmartLid is designed to easily transfer Magnetic Beads through a series of sample extraction steps.

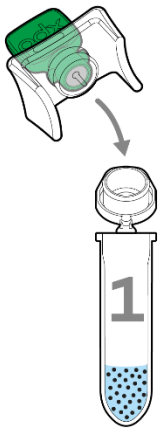
The SmartLid protocol repeats the following simple transfer process several times.



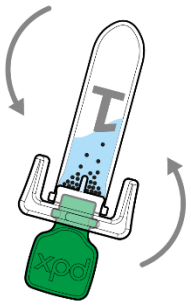
A powerful removable Magnetic Key is utilised to either capture or release the Beads.



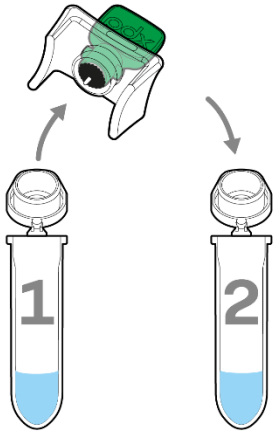
Residual buffer efficiently wicks away from the Beads limiting carry-over.



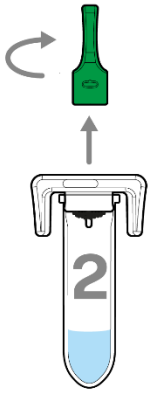
1 **Capture**: Insert SmartLid with Magnetic Key into tube 1.



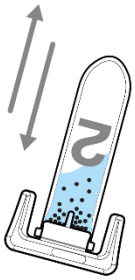
2 Invert the tube several times to collect the Beads until liquid is clear.



3 **Transfer**: Remove the SmartLid with collected Beads from tube 1 and place into tube 2.



4 **Release**: Remove the Magnetic Key to release the Beads.



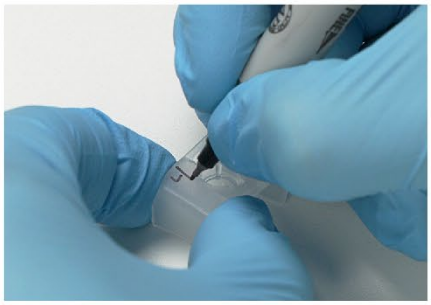
5 Resuspend the Beads through gentle mixing.

6 Beads with attached nucleic acids are successfully transferred to tube 2.

Multi-sample processing

Multiple samples can be processed using the SmartLid Rack and Smartlid Vortex Tool accessories.

Setup: Set out and prefill the required number of SmartLids and tubes. Labelling each SmartLid provides easy sample tracking throughout the process.



Mixing: The SmartLid Vortex Tool provides a convenient solution for simultaneously mixing up to 12 samples at a time.

Bead collection: Inverting the SmartLid Vortex Tool, with Magnetic Keys inserted, enables rapid and efficient Magnetic Bead collection.

7. Protocols for Extraction of DNA/RNA from Whole Blood

IMPORTANT Before Starting

1. Add molecular biology grade ethanol (>99%) to Wash Buffers BW-1 and BW-2 as indicated on the bottle. There is a box on the bottle label to check once this is completed.

2. Add Enzyme Powder BZ-L to Enzyme Buffer BZ-L as indicated on the bottle. There is a box on the bottle label to check once this is completed. Ensure as much powder as possible is recovered:

   i. Tap the base of the plastic ampule (containing Enzyme Powder BZ-L) on a hard surface multiple times to seat the powder in the bottom half.

   ii. Gently crack open the ampule and pour the entire contents into Enzyme Buffer BZ-L, again tapping the base of the ampule to empty as much powder as possible.

   iii. Close Enzyme Buffer BZ-L and pulse vortex (or shake) for 30 seconds to ensure complete resuspension.

   Note: It is important to use Enzyme Buffer BZ-L immediately or freeze for later use. We recommend aliquoting into smaller volumes prior to freezing, in order to limit the number of freeze-thaw cycles the buffer undergoes. Enzyme Buffer BZ-L has been validated to maintain performance for at least **3** freeze-thaw cycles.

3. Always ensure all buffers are liquid and homogeneous prior to use. In the case of visible precipitate, redissolve by warming and gentle mixing.

If using the SmartLid Blood DNA/RNA Extraction Kit for the first time, we recommend watching our instructional videos by visiting www.protondx.com or scanning the QR code (right).

Resources and Training Videos

SETUP for Multiple Extractions

1. Set up the required number of flip-cap tubes in a rack (Recommended: SmartLid Rack 100175), with different rows for **Lysis** (Row A), **Wash I** (Row B), **Wash II** (Row B) and **Elution** (Row D). For example, 6 extractions will require a total of 24 tubes, split in 4 rows of 6 tubes.

2. We recommend creating a master mix of **Lysis Buffer BL-1**, **Magnetic Beads**, and **Enzyme Buffer BZ-P** to ensure more consistent extractions. Prepare this master mix according to ratios in the table below, and multiply by the number of extractions. This will be needed for all protocols, including for extraction from gram-positive bacteria.

   Important: Always mix well (vortex) the Magnetic Beads before dispensing them into the master mix to ensure the correct concentration. Our Magnetic Beads settle quickly – please dispense immediately after vortexing.

Component	Target volume per tube	Volume for n extractions [1]
Lysis Buffer BL-1	600 µL	600 µL × **n**
Magnetic Beads	20 µL	20 µL × **n**
Enzyme Buffer BZ-P	20 µL	20 µL × **n**
Total volume [1]	640 µL	640 µL × **n**

[1] Use 10% overage calculation when making a master mix for use with multiple samples.

3. Prefill all tubes according to the table below:

   Important: Before dispensing the Lysis master mix, gently invert multiple times to insure an even dispersion of Magnetic Beads. Vigorous mixing or vortexing will create excessive bubbles/foam.

Tube/Step	Components and Volumes	
A: Lysis	If extracting from **viruses or gram-negative bacteria:** **640 µL** Lysis master mix	If extracting from **gram-positive bacteria:** **200 µL** Enzyme Buffer BZ-L [1]
B: Wash I	**500 µL** Wash Buffer BW-1	*(Ensure EtOH has been added to the bottle before use.)*
C: Wash II	**500 µL** Wash Buffer BW-2	*(Ensure EtOH has been added to the bottle before use.)*
D: Elution	**50 µL** Elution Buffer BE-1 or nuclease-free water	

[1] Ensure that Enzyme BZ-L has been added to Enzyme Buffer BZ-L as indicated on the bottle. Store in frozen aliquots when not in use.

LYSIS

Extracting circulating, viral, or gram-negative nucleic acids:

1. Add up to **100 µL** of sample to the lysis tube (prefilled with **640 µL** Lysis Buffer BL-1, Magnetic Beads, and Enzyme Buffer BZ-P master-mix).

2. Close with a SmartLid (without a Magnetic Key inserted) and briefly shake or vortex the tube (**1-2 seconds**) to ensure the sample and Lysis master-mix is sufficiently mixed.

3. Incubate the lysis tube (with SmartLid inserted) at **65°C** for **5 minutes**.

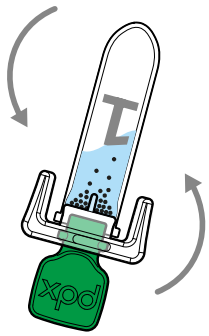
4. Temporarily remove the SmartLid and add **400 µL** of EtOH (>99%) to the lysed sample.

5. Return the SmartLid (still without the Magnetic Key) to the tube and vortex (or pulse-vortex) for **60 seconds**.

   Note: For this mixing/shaking step, and all to follow, the SmartLid Vortex Tool (100444) can be used to conveniently enable simultaneous mixing of up to 12 samples at a time. (See instructional video for more information at www.protondx.com or the QR code above.)

6. Insert a Magnetic Key into the SmartLid, turning it 90 degrees clockwise to lock, and invert the tubes several times to collect all Magnetic Beads onto the SmartLid.

   Note: This collection step is the longest of the entire protocol, due to the large volume and high viscosity of the Lysis master-mix. We recommend allowing the tube to remain upside down for **~30 seconds** after the first inversion, followed by multiple quicker (**~3-4 seconds each**) inversions to ensure all magnetic beads are collected.



Extracting nucleic acids from gram-positive bacteria:

1. Add up to **100 µL** of sample to the lysis tube (prefilled with **200 µL** Enzyme Buffer BZ-L).

2. Close with a SmartLid (without a Magnetic Key inserted) and briefly shake or vortex the tube (**1-2 seconds**) to ensure the sample and buffer is sufficiently mixed.

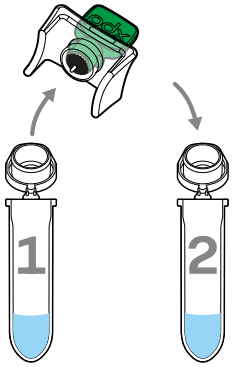
3. Incubate the lysis tube (with SmartLid inserted) at **37°C** for **20 minutes.**

4. Temporarily remove the SmartLid and add **640 µL** of Lysis master mix (See tables above. Before dispensing, ensure Magnetic Beads are evenly dispersed through gentle inversions.) Close again with the same SmartLid (still without a Magnetic Key) and briefly shake or vortex the tube (**1-2 seconds**).

5. Incubate the lysis tube (with SmartLid inserted) at **65°C** for **5 minutes**.

6. Follow steps 4-6 above from the **"Extracting circulating, viral, and gram-negative nucleic acids"** section before moving on to the Wash protocol below.

WASH



1. Transfer the SmartLid (with Magnetic Key INSERTED) from the lysis tube into the corresponding Wash I tube (containing **500 µL** of BW-1), ensuring it's firmly inserted.

   Note: If the Magnetic Key is not inserted during transfer of the SmartLid, the Magnetic Beads can fall off, and the captured nucleic acids will be lost.

2. Remove the Magnetic Key and vortex (or pulse-vortex) the tube for **60 seconds**, ensuring that all Magnetic Beads are fully resuspended.

3. Insert/lock the Magnetic Key into the SmartLid and invert the tube several times to collect all Magnetic Beads onto the SmartLid. Ensure the liquid is clear before proceeding.

   Note 1: It can help to initially shake the tube with the Magnetic Key inserted to first resuspend all Magnetic Beads, before gently inverting to collect the beads onto the SmartLid.

4. Repeat steps 1-3 for Wash II (containing **500 µL** of BW-2), transferring, resuspending, mixing, and again collecting the beads.

5. Once all washing steps are complete, and all Magnetic Beads are collected onto the SmartLid, remove the SmartLid and set it down (Magnetic Beads facing down) on a clean surface for **60 seconds** to **allow all EtOH to evaporate.**

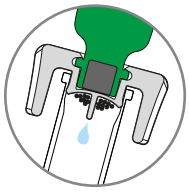
ELUTION

1. Once the evaporation step is complete, insert the SmartLid (with Magnetic Key still INSERTED) into the corresponding elution tube.

2. Remove the Magnetic Key and vortex the tube for **60 seconds**, ensuring that all Magnetic Beads are fully resuspended.

   Note: Due to the small volume of Elution Buffer BE-1, ensure all liquid is in the base of the tube before mixing. Alternatively, shaking instead of vortexing is acceptable for this step.

3. Insert/lock the Magnetic Key into the SmartLid. This time, due to the small liquid volume for the elution step, after gently inverting 1-2 times, **shake the tube to fully collect all Magnetic Beads.**



4. Flick down the tube (do not centrifuge) to collect as much elution volume as possible, discard the SmartLid and attached Magnetic Beads (all nucleic acids are now released into solution), and place the closed elution tube in a cold block or on ice until further use.

   Note: Retain the Magnetic Keys for future extractions. They can be reused indefinitely to limit disposable waste.

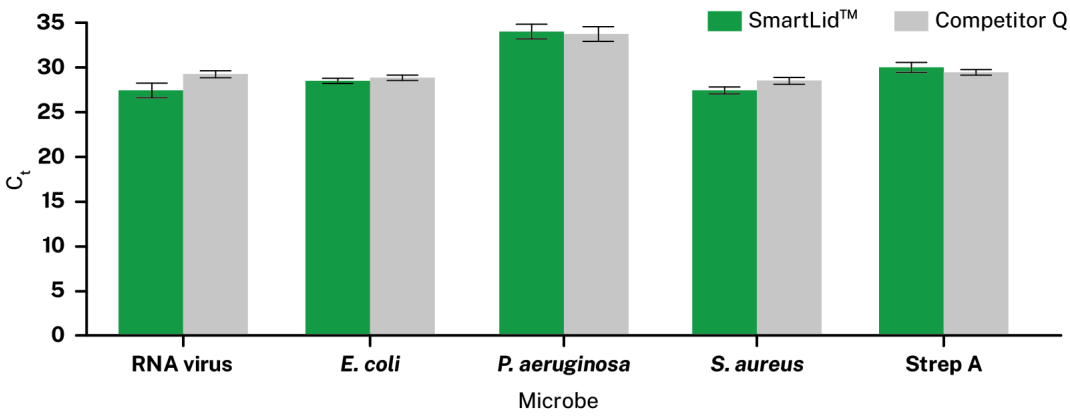
The purified and eluted nucleic acids are now ready for immediate use in downstream applications.

8. Troubleshooting

Problem	Possible Cause	Suggested Solution
Low nucleic acid yield or quality	Magnetic Bead concentration	Ensure Magnetic Beads are sufficiently mixed prior to dispense. Insufficient mixing can result in a Magnetic Beads concentration that is too low or too high. Our Magnetic Beads settle quickly – please dispense immediately after vortexing.
	Incomplete elution or insufficient elution volume	Increase elution volume (note: this will dilute the sample). We do not recommend eluting in less than 30uL.
		Heating the elution volume (prior to adding and mixing with the Magnetic Beads) may increase final yield.
	RNA/DNA is degraded	Maintain a sterile, nuclease-free environment while working to avoid any contamination.
		Make sure the elution is immediately processed or stored at -80°C after extraction is complete.
		Avoid repeated freeze/thaw cycles to preserve the RNA/DNA.
	Poor gram-positive extraction performance	Ensure Enzyme Powder BZ-L was added to Enzyme Buffer BZ-L before use. Once combined, Enzyme Buffer BZ-L must be stored frozen.
Low elution volume	When working with small volumes, liquid may adhere to the SmartLid due to surface tension	Flick down the tube to collect more elution volume from the SmartLid. Do not centrifuge tubes while SmartLid is inserted.
Magnetic Beads clumping	Dirty or viscous sample	Mix more vigorously and/or for longer periods of time.
		Increase wash buffer volume to dilute contaminants.
	Sample not fully digested	Ensure Enzyme Buffer BZ-P was added prior to the 5-minute incubation at 65°C.

9. Performance

Broad Microbe Compatibility



Extract a wide variety of microbes from whole blood with a single kit.

Figure 1. Extraction performance of contrived samples (100 µL of whole blood spiked with inactivated viral particles and live cultured bacterial cells) with analysis in RT-qPCR versus Competitor Q.

Rapid & Simple Protocols

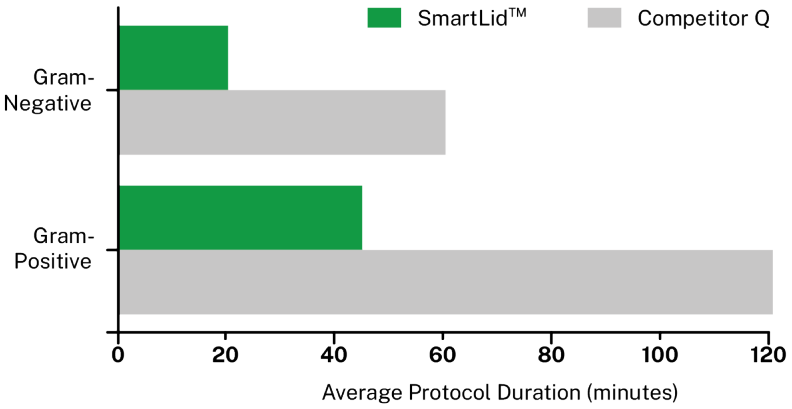


Figure 2. Comparison of average protocol time between the power-free SmartLid process and Competitor Q for both gram-negative and gram-positive bacteria.

High Yield & Purity

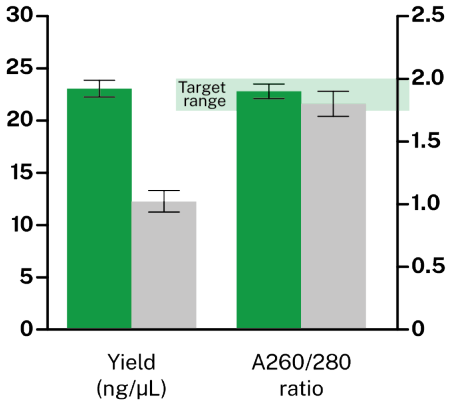


Figure 3. Relative nucleic acid (DNA) yield and purity for elutions extracted form whole blood spiked with live cultured *P. aeruginosa*.

Contamination-free Workflow

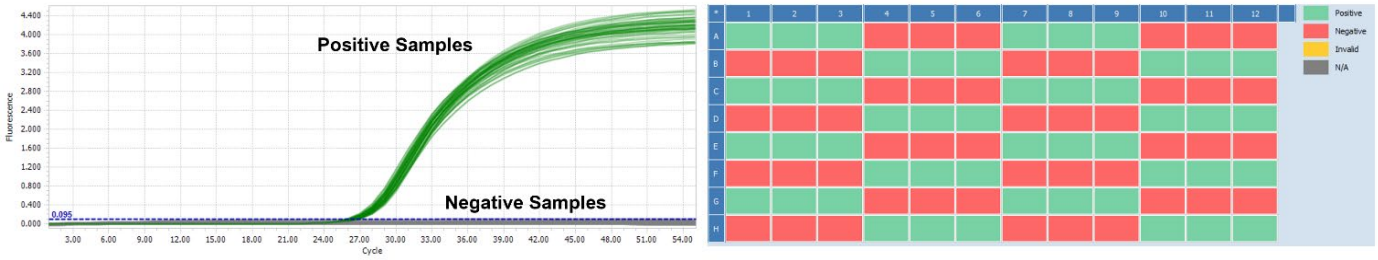


Figure 4. Alternating high-positive samples (spiked with 200 µL of 1×10^6 pfu/mL inactivated viral particles) and negative control samples were extracted in close proximity in a single batch. As shown by the raw RT-qPCR readout (LightCycler® LC96), all positive samples amplified while all negative samples did not amplify.

10. Ordering information

Catalogue No.	Item Name	Contents
100449	SmartLid Blood DNA/RNA Extraction Starter Pack	Everything needed to perform 50 extractions from whole blood, plus recommended accessories (100175, 100444)
100373	SmartLid Blood DNA/RNA Extraction Starter Kit	Same as Starter Pack (100449), excluding accessories
100374	SmartLid Blood DNA/RNA Extraction Refill Kit	Same as Starter Kit (100373), excluding reusable SmartLid Magnetic Keys
100375	SmartLid Blood DNA/RNA Extraction Trial Kit	Same as Starter Kit (100373), but for only 10 extractions
100175	SmartLid Rack	Accessory rack for processing 12 samples simultaneously
100444	SmartLid Vortex Tool	Accessory tool for vortexing 12 samples simultaneously
100173	SmartLid Shaker	Accessory shaker for mixing 12 samples simultaneously

11. Technical Support

For any questions or to report any issues, please contact support@protondx.com.

Answers to workflow questions may be found by watching the videos and reviewing the documentation linked to the QR Code below:

Resources and Training Videos