Luna Nanotech

SensoGOLD™ Pathogen Identification Kit

Target: *Enterococcus faecalis (EF)*

Color Code:

SG081-EF, SG083-EF

Product Manual

Version 3.1

The kit includes silica columns and reagents for the extraction of total bacterial RNA.

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Kit Contents

Component	36 Preps	72 Preps
Lysis Buffer	15 mL	24 mL
Wash Buffer 1	40 mL	80 mL
Wash Buffer 2	40 mL	80 mL
Water, nuclease-free	10 mL	10 mL
RNA Purification Columns	36	72
Collection Tubes (2 mL)	36	72
SensoGOLD™ Active Nucleic Acids Target: *Enterococcus faecalis* (***Tube AN***) *	36	72
SensoGOLD™ Nanoparticle Probes Target: *Enterococcus faecalis* (***Tube NP***) *	36	72
SensoGOLD™ (+) DNA Control Target: *Enterococcus faecalis*	5 mL	5 mL
SensoGOLD™ Activating Solution	1 mL	2 mL
SensoGOLD™ Thin Layer Chromatography (TLC) Plate	1	1
Foam Floating Rack	1	1

* ***SensoGOLD™ Active Nucleic Acids*** *and* ***SensoGOLD™ Nanoparticle Probes*** *are provided in freeze-dried powdered form in screw cap and regular cap 0.6 mL microcentrifuge tubes respectively. The tubes are color coded to match the target pathogen.*

Storage

SensoGOLD™ Active Nucleic Acids, **SensoGOLD™ Nanoparticle Probes** and **SensoGOLD™ (+) DNA Control** are stable for at least 1 year when stored at ≤4°C. For short-term storage (< 1 week), they can be kept at room temperature. All other kit components can be stored at room temperature.

Additional materials and equipment required

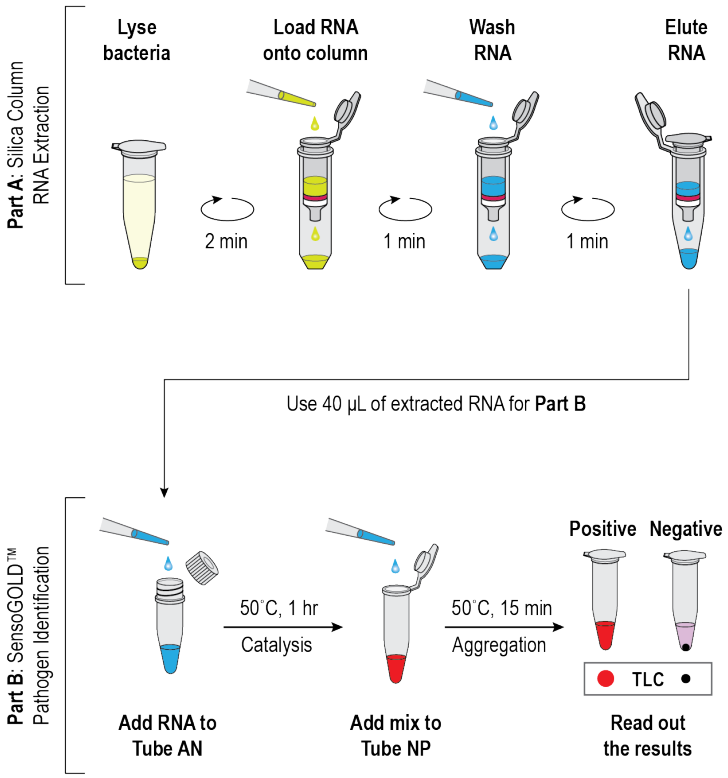
- TE Buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- Lysozyme (diluted in TE to 3 mg/mL)
- 2 M DTT or 14 M β-mercaptoethanol
- 100% Ethanol
- Pipettes, sterile nuclease-free tips
- 1.5 mL microcentrifuge tubes
- 50°C water bath or heating block
- Microcentrifuge (with 12,000g capability)
- Vortex (if not available, can be replaced with vigorous pipetting)
- Appropriate personal protective equipment

Kit Description

SensoGOLD™ Pathogen Identification Kits identify target pathogens based on their 16S rRNA molecular signatures. The protocol consists of 2 parts. In **Part A**, silica columns are used to extract and purify total RNA from overnight bacterial cultures. In **Part B**, **SensoGOLD™** reagents are used to identify the target bacteria using a simple colorimetric readout. Presence of target bacteria produces bright red color in ***Tube NP***, while absence of the target is indicated by dilute purple solution and dark precipitate at the bottom of the tube. If the storage of the results is required, the test solutions can also be spotted on the provided **thin layer chromatography** (**TLC**) plate. Larger red spots will be produced for positive target identification, while small dark spots will indicate negative detection.

This kit can also be combined with **SG081-BP** or **SG083-BP,** which include reagents targeting ***Universal Bacterial Pattern*** representing highly conserved 16S rRNA region. These probes will report positive detection in the presence of any bacterial species. Therefore **SensoGOLD™ Universal Bacterial Pattern Recognition Kit** can be used in parallel with pathogen-specific **SensoGOLD™ Pathogen Identification Kits** as an external positive control to confirm bacterial presence in the culture medium at sufficient concentration for positive **SensoGOLD™** detection.

Experimental Workflow



Experimental Setup Details

RNA extractions should be performed from bacteria cultured overnight, or until O.D. 600 between 1 and 6 is reached (~ 10^8-10^9 CFU/mL).

The kit includes reagents to be used for internal positive and negative control. For the negative control, nuclease free water is added to ***Tube AN*** instead of the extracted RNA. The negative control serves to ensure that **SensoGOLD™ Nanoparticle Probes** form controlled aggregates when the target pathogen is not present. For the positive control, **SensoGOLD™ (+) DNA Control** is added to ***Tube AN*** instead of the extracted RNA. Positive control is used to ensure that **SensoGOLD™ Active Nucleic Acids** components are functioning properly and able to disrupt nanoparticle aggregation and cause appropriate solution color change in response to the presence of the target pathogen. Both of the internal positive controls are only included in **Part B** of the protocol, and therefore do not need to be passed through RNA isolation in **Part A**.

If a culture of known target bacteria is available, it can be included as an external positive control. In this case it needs to be processed both through **Part A** and **Part B** of the protocol.

For each procedure testing ***N*** samples, the following number of silica columns and tubes need to be prepared. External positive control of known cultured target bacteria is included in this number. For example, when testing 3 unknown samples and 1 external positive control, ***N* = 3+1 = 4**.

Kit Component	**Number**
RNA Purification Columns	*N*
Collection Tubes	*N*
Tube AN	*N* + 2 *
Tube NP	*N* + 2 *

* *The extra 2 tubes are included to be used for the internal positive and negative controls.*

Important Notes

The **Lysis Buffer** contains 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.

For most bacteria overnight cultures in **lysogeny broth** (**LB**) culture medium will result in sufficient bacterial counts (O.D. 600 between 1 and 6 corresponding to 10^8-10^9 CFU/mL) to produce strong positive signal with the **SensoGOLD™ Pathogen Identification Kit**. If the overnight **LB** culture produces insufficient O.D. measurements, culture the bacteria in alternative media such as **tryptic soy broth** or **brain-heart infusion** medium.

In certain cases preparations from highly concentrated overnight cultures of such bacteria as *Klebsiella spp*. and *Pseudomonas spp*., which have a tendency to form biofilms, can be very viscous and therefore difficult to pipette and can potentially clog the membrane. This can be overcome by diluting the overnight cultures by a factor of 4-10 prior to the RNA extraction. While this will somewhat decrease the total yield of purified RNA, the extracted amount should still be sufficient for testing with the **SensoGOLD™ Pathogen Identification Kit**.

Part A. Bacterial Total RNA Purification Protocol

Before setting up the experiment, grow bacteria overnight. O.D. 600 absorbance measurement should be in the range between 1 and 6, which corresponds to ~10^8-10^9 CFU/mL.

If precipitate is observed in the Lysis Buffer or Wash Buffer solutions, re-dissolve it by warming the solution to 37°C. Cool the solution back to room temperature before use.

Within 30 min of starting the RNA extraction, prepare the following reagents:

- Make **Working Lysis Buffer** by adding 20 µL of 2 M DTT or 14 M β-mercaptoethanol solution to each 1 mL of Lysis Buffer used. Each 1 mL of **Working Lysis Buffer** is sufficient for 4 reactions.
- Make 3 mg/mL of **Lysozyme Solution** by dissolving lysozyme in the appropriate volume of TE buffer.

Step	Procedure
1	Transfer 1 mL of bacterial culture (~10^9 CFU/mL) into 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	To each tube add 100 µL of **Lysozyme Solution**. Pipette up and down until homogenous suspension of cells is achieved.
3	Incubate the cell suspension for 5 min at room temperature.
4	Add 250 µL of the **Working Lysis Buffer**. Vortex vigorously for a few seconds.
5	Add 350 µL of 100% Ethanol. Invert a few times to mix.

6	Transfer 700 µL of the solution onto the **RNA Purification 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 volume is more than 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. If the liquid still remains, this might be due to the column clogging by the cellular and extracellular residue. See the note on Page 7 for steps to overcome this problem.*
7	Add 700 µL of **Wash Buffer 1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
8	Add 700 µL of **Wash Buffer 2** 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 **RNA Purification Column** into a new 1.5 mL microcentrifuge tube.
11	Pipette 100 µL of included **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the RNA. Keep the flow-through that contains the product and discard the column. *NOTE: 40 µL of the extracted RNA solution will be used in Part B. The remaining volume can be used for other applications, such as RT-qPCR or sequencing.*

Part B. SensoGOLD™ Bacterial Pathogen Identification

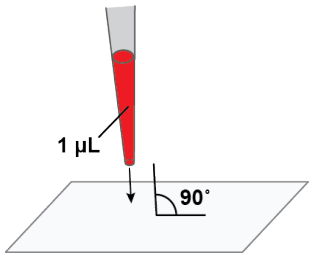
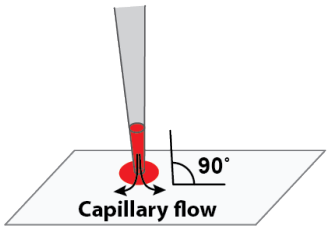
Accurate pipetting is critical when working with **SensoGOLD™** reagents. Please make sure that you use well-calibrated pipettes and proper pipetting techniques when performing the protocol.

*NOTE: If (*optional*) Step 9 is included, it must be performed before Step 10. 1 min 1,000g centrifugation carried out at Step 10 will cause aggregated nanoparticles to crush out of solution and prevent the formation of proper spots on the **TLC plate**.*

Step	Procedure
1	Centrifuge the required number (see **Page 5** for experimental setup details) of **SensoGOLD™ Active Nucleic Acid** (***Tube AN***) and **SensoGOLD™ Nanoparticle Probes** (***Tube NP***) component tubes at 12,000g for 1 min to collect freeze-dried powder at the bottom of the tubes. Label appropriately. *NOTE: If testing for multiple targets is parallel by combining multiple* **SensoGOLD™ Bacterial Pathogen Identification Kits**, *make sure that color-coded labels match for* ***Tubes AN*** *and* ***Tubes NP***.
2	• For each sample add 40 µL of the RNA solution extracted in **Part A** to ***Tube AN***. If external positive control of known cultured bacterial targets is included, it is processed in the same way as the unknown samples. • For the internal negative control, add 40 µL of **nuclease-free water** to ***Tube AN***. • For the internal positive control, use 40 µL of **SensoGOLD™ (+) DNA Control** solution to ***Tube AN***.
3	Add 20 µL of **SensoGOLD™ Activating Solution** to each ***Tube AN***. Vortex vigorously for a few seconds.
4	Incubate the tubes at 50°C for 1 hour. After incubation, pulse-spin the tubes to collect all of the liquid at the bottom.
5	Transfer 20 µL from each tube to a new ***Tube NP***.

6	Vortex vigorously for a few seconds to ensure that all of the gold nanoparticle are fully resuspended (indicated by the red solution color).
7	Centrifuge the tubes at 100g for 10 sec to collect solution at the bottom. *NOTE: **Do not** centrifuge at higher speeds or increase the spin time. Aggregated nanoparticles can crash out of solution and prevent formation of proper spots on **TLC plates** in (*optional*) Step 9.*
8	Incubate the tubes at 50°C for 15 min.
9	(*Optional*) If storage of the results for future reference is required, follow the instructions on the next page to spot 1 µL of the solution on the provided **TLC plate**. Larger red spots indicate positive pathogen identification. This should be observed for the **SensoGOLD™ (+) DNA Control** positive control. Smaller dark spots indicate a negative detection. This should be observed for the **nuclease-free water** negative control. *NOTE: if this (optional) step is included, it must be performed before Step 10.*
10	Centrifuge the tubes at 1,000g for 1 min. Uniform red-colored solution indicates positive pathogen identification. This should be observed for the **SensoGOLD™ (+) DNA Control** positive control. Clear or lightly purple solution with nanoparticles clumped together at the bottom of the tube indicates the target pathogen is not present. This should be observed for the **nuclease-free water** negative control.

Instructions for TLC Plate Spotting

Aspirate 1 µL of the solution from ***Tube NP*** into a 10 µL tip. Position the tip at 90° angle to the **TLC plate** and lower it down slowly until the tip lightly touches the surface of the plate.	
At this stage the liquid will begin flowing onto the **TLC plate** due to capillary flow. Keep the tip touching the plate at 90° angle and let capillary flow to transfer the solution from the tip to the plate. *NOTE 1: Do not actively dispense the liquid from the pipette, since this will disrupt proper spot formation.* *NOTE 2: If the flow stops before all the liquid has transferred onto the **TLC plate**, lightly press the dispense lever on the pipette until the liquid again touches the surface of the plate and capillary flow resumes, at which stage release the level and let capillary forces take over.*	

Troubleshooting

Problem	Possible causes and solutions
Column clogging	**Bacterial culture too concentrated.** This can occur for highly concentrated cultures of biofilm-forming bacteria (e.g., *Klebsiella* and *Pseudomonas spp.*). Dilute bacterial culture 4-10 times before performing RNA extraction.
Internal negative control produces red solution and larger red spots on the TLC plate	***Nanoparticles failed to aggregate.*** **Excessive volume was pipetted into the tube.** Use properly calibrated pipettes and proper pipetting techniques to ensure that appropriate volumes of reagents are added in the SensoGOLD™ protocol. **Freeze-dried pellet not fully resuspended.** Make sure that all AN and NP tubes are centrifuged before they are open to collect the powdered reagents at the bottom. Failure to do so might result in the loss of powdered reagents stuck to the tube lid. **Nanoparticles fail to aggregated due to cellular residue not removed due to column clogging.** Dilute bacterial culture 4-10 times before the Total Bacterial RNA extraction step.
Internal positive control produces light purple solution with precipitates and small dark spot on the TLC plate	***Active Nucleic Acids catalysis failed*** **Insufficient volume was pipetted into the tube.** Use properly calibrated pipettes and proper pipetting techniques to ensure that appropriate volumes of reagents are added during the SensoGOLD™ protocol.
Known target bacteria external positive control incorrectly reported as negative.	**Insufficient RNA extraction** Extend the bacterial culture incubation time to increase bacterial concentration. Use alternative media (e.g., tryptic soy broth, brain-heart infusion medium) to improve bacterial growth.

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Lysis Buffer		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 intended solely for research use only. Full material safety data sheet document is available at **www.lunanano.com**.

For further information about the technology behind the **SensoGOLD™ Pathogen Identification Kits** please refer to **www.lunanano.com.**

Notes