LSBio
an Absolute Biotech Company

Pig Rotavirus Group Specific Antigen ELISA Kit (Sandwich)

User Manual

Catalog No. LS-F10367

It is important that you read this entire manual carefully before starting your experiment.

This kit is for **Research Use Only**.
This kit is not approved for use in Humans or for clinical diagnosis.

SAMPLE ONLY

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ASSAY SPECIFICATIONS

Target:	Rotavirus Group Specific Antigen
Specificity:	This kit is for the detection of Pig Rotavirus Group Specific Antigen. No significant cross-reactivity or interference between Rotavirus Group Specific Antigen and analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.
Sample Types:	This kit is recommended for use with Pig feces. Use with other sample types is not supported.
Detection:	Colorimetric - 450nm
Measurement:	Qualitative
Detection Range:	Positive / Negative
Performance:	Intra-Assay CV≤15%; Inter-Assay CV≤15%
Limitations:	This kit is for **Research Use Only** and is not intended for diagnostic use. This kit is not approved for use in humans or for clinical diagnosis.

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ASSAY PRINCIPLE

This assay is based on the sandwich ELISA principle. Each well of the supplied microtiter plate has been pre-coated with a target specific capture antibody. Controls or samples are added to the wells and the target antigen binds to the capture antibody. Unbound Control or sample is washed away. A Horseradish Peroxidase (HRP)-conjugated detection antibody is then added which binds to the captured antigen. Unbound detection antibody is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. The OD of an unknown sample can then be compared to the OD of the positive and negative controls in order to determine the presence or absence of the antigen.

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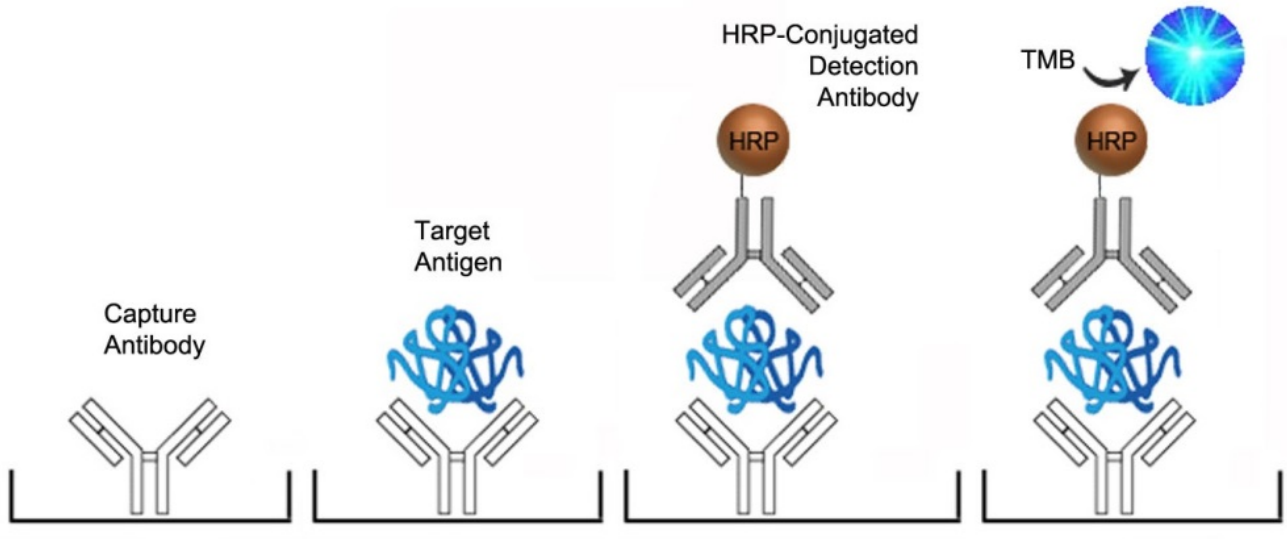
ASSAY PRINCIPLE IMAGE



KIT COMPONENTS

Component	Quantity
Coated 96-well Strip Plate	1
Positive Control	1 vial x 800 µl
Negative Control	1 vial x 800 µl
HRP-conjugate Antibody	1 vial x 6 ml
Wash Buffer	1 vial x 6 ml
Substrate A	1 vial x 5 ml
Substrate B	1 vial x 5 ml
Stop Solution	1 vial x 5 ml
Plate Sealers	4
Instruction Manual	1

KIT STORAGE

The unopened kit can be stored at 2-8°C through the expiration date. Once opened, the kit can be stored at 2-8°C for2 weeks. Unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air.

OTHER REQUIRED SUPPLIES

- Microplate reader with 450nm wavelength filter, with the correction wavelength set at 540 nm – 570 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- High-precision pipette and sterile pipette tips
- Eppendorf tubes
- 37°C incubator
- Deionized or distilled water
- Absorbent paper

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SAMPLE COLLECTION

This assay is recommended for use with serum or plasma. No other sample types are supported. The sample collection protocols below have been provided for your reference.

Breast Milk - Centrifuge samples for 20 minutes at 1000×g to remove particulates. Collect the supernatant for assaying.

Cell Lysates - Collect and pellet the cells by centrifugation and remove the supernatant. Wash the cells 3 times with PBS* then resuspend in PBS*. Lyse the cells by ultrasonication 4 times. Alternatively freeze the cells to -20°C and thaw to room temperature 3 times. Centrifuge at 1500×g for 10 minutes at 2 - 8°C to remove cellular debris. Collect the supernatant for assaying.

Erythrocyte Lysates - Centrifuge whole blood for 20 minutes at 1000×g to pellet the cells and remove the supernatant. Wash the cells 3 times with PBS* then resuspend in PBS*. Freeze (-20°C)/thaw (room temperature) the cells 3 times. Centrifuge at 5,000×g for 10 minutes at 2-8°C to remove cellular debris. Collect the supernatant for assaying. Erythrocyte lysates must be diluted with Sample Diluent before running.

Feces – Direct collect water liquid for detection. If no fluid, dilute samples 20-30 times with normal saline. Centrifuge at 1,000 rpm for 1-2 minutes at 2 - 8°C to remove cellular debris. Collect the supernatant for assaying immediately or aliquot and store at -20°C. Avoid repeat freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. Collect the supernatant for assaying.

Platelet-Poor Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. It is recommended that samples should be centrifuged for 10 minutes at 10,000×g for complete platelet removal. Collect the supernatant for assaying.

Sperm and Seminal Plasma – Allow semen to liquefy at room temperature or 37°C. After liquefaction, centrifuge at 2,000×g for 10-15 minutes. Collect seminal plasma supernatant for assaying. Wash the precipitated protein 3 times with PBS* then resuspend in PBS*. Lyse the

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cells by ultrasonication then centrifuge at 2,000×g for 10-15 minutes. Collect the supernatant for assaying.

Serum - Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant for assaying.

Tissue Homogenates–Because preparation methods for tissue homogenates vary depending upon tissue type, users should research tissue specific conditions independently. The following is one example only. Rinse tissues in PBS* to remove excess blood and weigh before homogenization. Finely mince tissues and homogenize them in 5-10mL of PBS*with a glass homogenizer on ice. Lyse the cells by ultrasonication or freeze (-20°C)/thaw (room temperature) 3 times. Centrifuge homogenate at 5000×g for 5 minutes. Collect the supernatant for assaying.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter and collect the supernatant for assaying.

Cell culture supernatants, cerebrospinal, follicular, and lung lavage fluids, saliva, sweat, tears, and other biological fluids - Centrifuge samples for 20 minutes at 1000×g to remove particulates. Collect the supernatant for assaying.

* 1xPBS (0.02mol/L pH7.0-7.2)

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SAMPLE COLLECTION NOTES

1. LSBio recommends that samples are used immediately upon preparation. Alternatively, samples stored at 2-8°C should be used within 5 days. For long-term storage sample aliquots should be prepared and stored at -20°C if used within 1 month, or -80°C if used within 6 months. Long term storage can result in protein degradation and denaturalization, which may result in inaccurate results.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. LSBio is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples use with the kit.

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REAGENT PREPARATION

Bring all reagents to room temperature (18-25°C) before use.

REAGENT PREPARATION NOTES

1. All solutions prepared from concentrates are intended for one-time use. Do not reuse solutions.
2. Reagents may adhere to the tube wall or cap during transport so centrifuge tubes briefly before opening.
3. All solutions should be gently mixed prior to use.
4. To minimize imprecision caused by pipetting, ensure that pipettes are calibrated. Pipetting volumes of less than 10 µL is not recommended.
5. Substrate solutions are easily contaminated so sterility precautions should be taken. Substrate Solution should also be protected it from light.
6. Do not substitute reagents from one kit lot to another. Use only those reagents supplied within this kit.
7. Due to the antigen specificity of the antibodies used in this assay, native or recombinant proteins from other manufacturers may not be detected by this kit.

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ASSAY PROCEDURE

Bring all reagents and samples to room temperature without additional heating and mixed thoroughly by gently swirling before pipetting (avoid foaming). Prepare all reagents and samples as directed in the previous sections.

1. Set a Blank well without any solution.
2. Set three **Negative Control** and two **Positive Control** wells.
3. Add 50 µl of **Positive/Negative Control** or **Sample** per well then add 50 µl of **HRP-conjugate** to each well (excluding the Blank well), cover with a plate sealer, and incubate for 15 minutes at room temperature.
4. Aspirate the liquid from each well and wash 1 time with 50 µl of **Wash Buffer**. Wash an additional 10 times by adding approximately 250 µl of **ddH_20** using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Allow each wash to sit for 30 seconds before completely aspirating. After the last wash, aspirating remove any remaining Wash Buffer then invert the plate and tap against clean absorbent paper.
5. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well, mix gently, and incubate **in the dark** for 10 minutes at room temperature.
6. Add 50 µl of **Stop Solution** to each well. The blue color will change to yellow immediately. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The Stop Solution should be added to wells in the same order and timing as was the substrate solution.
7. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm.

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ASSAY PROCEDURE NOTES

1. **ELISA Plate**: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be placed in a sealed bag containing desiccant, and stored at 4°C.
2. **Solutions**: To avoid cross-contamination, change pipette tips between addition of each sample and between reagent additions. Also, use separate reservoirs for each reagent.
3. **Applying Solutions**: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
4. **Assay Timing**: The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
5. **Incubation**: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
6. **Washing**: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
7. **Controlling Substrate Reaction Time**: After the addition of the Substrates, periodically monitor the color development. Stop color development before the color become too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance reading.
8. **Reading**: The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any

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residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.

9. **Reaction Time Control**: Control reaction time should be strictly followed as outline.
10. **Stop Solution**: The Stop Solution is contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face and clothing.
11. **Mixing**: During incubation times, the use of a micro-oscillator at low frequency is recommended. Sufficient and gentle mixing is particularly important in producing reliable result.
12. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the supplied instructions.
13. Due to inter- and intra-assay variability, it is recommended that appropriate carry over controls be included between assays.
14. Prior to running valuable samples LSBio recommends that the user runs a preliminary experiment using the supplied controls in order to validate the assay.
15. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
16. The kit should not be used beyond the expiration date on the kit label.

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ASSAY PROCEDURE SUMMARY

Prepare all reagents, samples and controls.

Set a Blank well with no solution.

Set 3 **Negative Control** and 2 **Positive Control** wells.

Add 50 µl of **Positive/Negative Controls,** or **Sample** and 50 µl of **HRP-conjugate** to each well and incubate for 15 minutes at room temperature.

Aspirate and wash 1 time with **Wash Buffer** the 10 times with ddH_20.

Add 50 µl of **Substrate A** and 50 µl **Substrate B** to each well and incubate in the dark for 10 minutes at room temperature.

Add 50 µl of **Stop Solution**.

Read immediately at 450nm.

CALCULATION OF RESULTS

For calculating the valence of Pig Rotavirus Group Specific Antigen, compare the O.D. values of the Sample well with the positive and negative control wells.

A cutoff value was defined as the average Negative Control value plus 0.1.

If the average value of $O.D._{negative} < 0.05$, calculate it as 0.05.

1. The negative control O.D. value should be less than 0.1, and the positive control O.D. value should be greater than 0.7. Or else, repeat the test.

2. Determine the results:

    - While $O.D._{sample} <$ Cutoff Value: Negative
    - While $O.D._{sample} \geq$ Cutoff Value: Positive

3. If the $O.D._{sample}$ is close to the Cutoff Value, we recommend repeating the experiment.

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Low signal	Too brief incubation times.	Ensure sufficient incubation time.
	Incorrect assay temperature.	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes.	Check pipettes and ensure correct preparation.
	Improper dilution.	
Deep color but low value	Plate reader settings not optimal.	Verify the wavelength and filter setting in the plate reader.
		Open the Plate Reader ahead to pre-heat.

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TROUBLESHOOTING GUIDE (CONTINUED)

Problem	Possible Cause	Solution
Large CV	Inaccurate pipetting.	Check pipettes.
High background	Concentration of detector too high.	Use recommended dilution factor.
	Plate is insufficiently washed.	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer.	Make fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit.	All the reagents should be stored according to the instructions.
	Stop solution not added.	Stop solution should be added to each well before measurement.

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Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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