RBD-LuciaV5 (P.1)

Soluble SARS-CoV-2 Gamma variant (P.1) Spike RBD protein fused to Lucia luciferase

Catalog code: rbd-lucia-v5

https://www.invivogen.com/p1-rbd-lucia

For research use only, not for diagnostic or therapeutic use

Version 23A11-MM

PRODUCT INFORMATION

Contents

- **50 µg of RBD-LuciaV5 (P.1)** (provided lyophilized)
- **1.5 ml endotoxin-free water**
- **1 tube of QUANTI-Luc™ 4 Reagent**, a Lucia luciferase detection reagent (sufficient to prepare 25 ml). Store at -20°C. Avoid repeated freeze-thaw cycles. This product is photosensitive and should be protected from light.

Protein construction: RBD [R319-F541] from the Gamma variant (P.1) Spike protein with a C-terminal Lucia luciferase reporter and histidine tag.
Sequence: GISAID EPI_ISL_811149 (codon optimized)
Origin: Gamma Variant (P.1 lineage) | Brazilian origin
Tag: 6 x Histidine tag
Total protein size: 461 amino acids (including Lucia luciferase)
Molecular weight: ~52 kDa
Purification: IMAC
Purity: >90% (SDS-PAGE)
Formulation: 0.2 µm filtered solution in a sodium phosphate buffer with 1% Bovine Serum Albumin (BSA), saccharose, and stabilizing agents.

Storage and stability

- Product is shipped at room temperature. Upon receipt, store lyophilized protein and QUANTI-Luc™ 4 Reagent at -20°C.
- Reconstituted protein is stable for 1 month when stored at 4°C and for 1 year when aliquoted and stored at -20°C.
- After preparation, QUANTI-Luc™ 4 Reagent working solution is stable for 48 hours at 4°C and 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles. Protect from light.

Quality control

- The size and purity of the protein has been confirmed by SDS-PAGE.
- RBD-LuciaV5 (P.1) has been functionally validated by a luciferase-based ELISA using clinically relevant anti-Spike mAbs.
- Absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and TLR4 cellular assays.

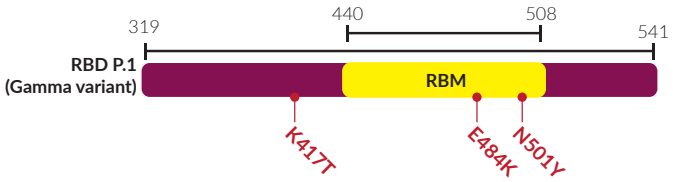
PRODUCT DESCRIPTION

RBD-LuciaV5 (P.1) (~52 kDa) is a soluble fusion protein composed of the Receptor Binding Domain (RBD) from the SARS-CoV-2 Gamma variant (P.1) Spike protein fused to a C-terminal Lucia luciferase reporter. RBD-LuciaV5 (P.1) has been generated by recombinant DNA technology, produced in CHO cells, and purified by IMAC (Immobilized Metal Affinity Chromatography) using a C-terminal histidine tag.
Spike (S) is a structural glycoprotein expressed on the surface of SARS-CoV-2. It mediates viral entry and membrane fusion of target cells upon binding to the host receptor ACE2[1].

SARS-CoV-2 Spike RBD

RBD-LuciaV5 (P.1) contains the Spike RBD from the SARS-CoV-2 Gamma variant (P.1), first reported in Brazil in December 2020. This variant is characterized by the presence of a number of mutations within the Spike RBD coding region, which are of concern[2].

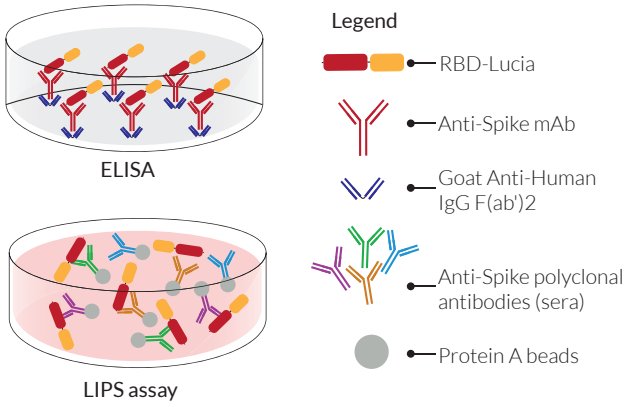
- K417T, E484K, N501Y



APPLICATIONS

RBD-LuciaV5 (P.1) has been specifically designed to assess the binding affinity of anti-Spike antibodies using either a solid-phase assay, ELISA, or the solution phase assay LIPS (luciferase immunoprecipitation systems).

- **ELISA**: the C-terminal Lucia luciferase tag provides a larger dynamic range than the commonly used HRP detection.
- **LIPS**: for the detection of antibodies, against both linear and conformational epitopes, in the sera of recovered COVID-19 patients and/or vaccinees[3]. *See the otherside for a detailed protocol.*



1. **Hoffmann M. *et al.*, 2020.** SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 181:1-16. 2. **Faria, N.R.*et al.* 2021.** Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science. doi:10.1126/science.abh2644. 3. **Haljasmagi, L. *et al.* 2020.** LIPS method for the detection of SARS-CoV-2 antibodies to spike and nucleocapsid proteins. Eur J Immunol 50, 1234-1236.

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METHODS

RBD-LuciaV5 (P.1) resuspension (100 µg/ml)

Note: Ensure you see the lyophilized pellet before resuspension.

- Add 500 µl of endotoxin-free water to the vial and gently pipette until completely resuspended.
- Prepare aliquots and store at -20 °C until required.

Luciferase-based ELISA protocol

Below is an optimized protocol for using InvivoGen's RBD-Lucia in a luciferase-based ELISA to measure the binding of monoclonal antibodies (mAb).

Note: This protocol is not optimized for sera testing.

Preparation of the plate

1. Prepare a coating solution by diluting a Fab'2 Anti-Fc (appropriate to the test mAb isotype) at 2 µg/ml in 50 mM carbonate-bicarbonate buffer.
2. Distribute 50 µl per well of a 96-well opaque MaxiSorp plate.
3. Incubate overnight at room temperature.
4. Discard the coating Anti-Fc solution.
5. Add 200 µl of PBS + 3% BSA (blocking solution).
6. Incubate for 2 hours at 37°C.
7. Discard the blocking solution.

Note: the plate may be stored at -20°C until further use.

Preparation of RBD-LuciaV5 (P.1) protein

1. Thaw an RBD-LuciaV5 (P.1) (100 µg/ml) aliquot
2. Prepare a 1 µg/ml working solution of RBD-LuciaV5 (P.1) in phosphate-buffered saline (PBS) + 0.05% Tween + 1% BSA.

Sample preparation

1. Prepare a solution of the test antibody in PBS + 0.05% Tween + 1% BSA.
2. Prepare solutions of the positive and negative control antibodies (e.g. Anti-CoV2RBD-imd-mIgG2a and Anti-βGal-mIgG2a, respectively) in PBS + 0.05% Tween + 1% BSA.
3. Further dilute the antibodies 1:2 or 1:3 in PBS + 0.05% Tween + 1% BSA.

General ELISA protocol

1. Distribute 50 µl of prepared samples (diluted antibody solutions) per well of the pre-coated MaxiSorp plate.
2. Add 50 µl/well of the RBD-Lucia protein working solution (1 µg/ml).
3. Incubate for 2 hours at 37°C.
4. Thoroughly wash (at least 3 times) the plate using 200 µl/well of PBS + 0.05% Tween.
5. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the enclosed data sheet.
6. Set the luminometer with the following parameters: end-point measurement with a 4 second start time and 0.1 second reading time.
7. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution to each well.
8. Proceed with the measurement.

LIPS protocol

Below is an optimized protocol for using InvivoGen's RBD-Lucia in a LIPS assay to measure the antibody response in recovered patient or vaccinee serum. This protocol has been specifically designed using Protein A agarose beads. If using other types of Protein A beads (e.g. magnetic beads), the protocol will need to be adjusted accordingly.

Preparation of RBD-LuciaV5 (P.1) protein

1. Thaw an RBD-LuciaV5 (P.1) (100 µg/ml) aliquot
2. Prepare a 10 µg/ml working solution of RBD-LuciaV5 (P.1) in phosphate-buffered saline (PBS) + 0.05% Tween + 1% BSA.

Sample preparation

- **Positive & negative controls**

1. Prepare 100 µg/ml solutions of the positive and negative control antibodies (e.g. Anti-CoV2RBD-imd-mIgG2a and Anti-βGal-hIgG1, respectively) in PBS.
2. Further dilute the antibodies 1:2 (50 µg/ml) in human serum (free of previous SARS-CoV-2 infection).

- **Sample preparation (patient/vaccinee serum samples)**

1. Heat the sample at 56°C for 30 minutes.

General LIPS protocol

1. Prepare Protein A beads according to the manufacturers protocol.
2. Equilibrate the Protein A beads in PBS and make a homogenous 30% suspension.
3. In a 96-well PCR plate (conical-bottom) add:
   a. 6µl of pre-equilibrated Protein A bead suspension
   b. 10 µl of RBD-LuciaV5 protein working solution (10 µg/ml)
   c. 1 µl of prepared sample ***(antibody controls or recovered patient/vaccinee serum (diluted in 40 µl PBS + 0.05% Tween + 1% BSA)***
4. Incubate at room temperature for 2 hours with orbital shaking.
5. Purify and thoroughly wash (at least 6 times) RBD-Lucia-antibody complexes bound to the Protein A beads.

Note: Depending on the type of Protein A beads used, this step can be performed by centifugation, magnetic rack, or as tested by InvivoGen, by transferring to a Polyvinylidene Fluoride (PVDF) plate and using vacuum suction.

6. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the enclosed data sheet.
7. Set the luminometer with the following parameters: end-point measurement with a 4 second start time and 0.1 second reading time.
8. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution to each well.
9. Proceed with the measurement.

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™ 4 Lucia/Gaussia	rep-qlc4lg1
Anti-Spike-RBD-hIgG1	srbd-mab1
Anti-Spike-RBD-mIgG2a	srbd-mab10
Anti-CoV2RBD-cas-mIgG2a	srbdc3-mab10
Anti-CoV2RBD-imd-mIgG2a	srbdc4-mab10
Anti-CoV2RBD-bam-mIgG2a	srbdc5-mab10
Anti-CoV2RBD-ete-mIgG2a	srbdc6-mab10
Anti-βGal-hIgG1	bgal-mab1
Anti-βGal-mIgG2a	bgal-mab10

Note: *For more products related to COVID-19 research, please visit our website https://www.invivogen.com/covid-19*

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QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

https://www.invivogen.com/quanti-luc

For research use only

Version 23A16-MM

PRODUCT INFORMATION

Contents

- 1 tube of **QUANTI-Luc™ 4 Reagent (20X)**

One tube of QUANTI-Luc™ 4 Reagent is sufficient for 5 x 96-well plates (25 ml standard Flash/end-point detection).

Note: This sample cannot be sold separately from the QUANTI-Luc™ 4 Lucia/Gaussia kit.

QUANTI-Luc™ 4 Lucia/Gaussia comprises two liquid components:

- QUANTI-Luc™ 4 Reagent 20X (coelenterazine substrate)
- QUANTI-Luc™ 4 Stabilizer 25X (optimized Glow assay reagent)

Find more information at https://www.invivogen.com/quanti-luc.

Storage and Stability

– Store QUANTI-Luc™ 4 Reagent at -20°C for up to 12 months.
– After preparation, the working solution is stable for 48 hours at 4°C and 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.
Note: This product is photosensitive and should be protected from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
– Physicochemical characterization (pH, appearance).
– Functional assays using recombinant Lucia protein or reporter cells.

DESCRIPTION

QUANTI-Luc™ 4 Reagent is a component of the QUANTI-Luc™ 4 Lucia/Gaussia kit. It contains the coelenterazine substrate for the detection of secreted Lucia or Gaussia activity in live-cell supernatants, and of intracellular Renilla after cell lysis. The light signal produced correlates to the amount of luciferase protein expressed. It is quantified using a luminometer and expressed as relative light units (RLUs).

METHODS

Preparation of QUANTI-Luc™ 4 Reagent working solution (1X):

1. Dilute the total volume of the 20X tube (1.25 ml) of Reagent into 23.75 ml of sterile water to obtain 25 ml of working solution.
2. Vortex **very briefly** (a few seconds).
3. Use the working solution immediately or store until required for use. QUANTI-Luc™ 4 Reagent working solution can be stored for 48 hours at 4°C or 1 month at -20°C.

Flash detection of luciferase activity from cell culture medium:

To obtain **end-point readings** using a luminometer **with an injector**.
1. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
2. Pipet 20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with QUANTI-Luc™ 4 Reagent 1X and proceed **immediately** with the measurement.

To obtain **end-point readings** using a luminometer **without injectors**.
1. Set the luminometer with a 0.1 second reading time.
2. Pipet 20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ 4 Reagent 1X to each well or tube.
4. Gently tap the plate several times to mix (do **not** vortex).
5. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Cat. Code
QUANTI-Luc™ 4 Lucia/Gaussia Kit	
500 tests	rep-qlc4lg1
2 x 500 tests	rep-qlc4lg2
5 x 500 tests	rep-qlc4lg5

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