

RBD-LuciaV3 (B.1.351)

Soluble SARS-CoV-2 Beta variant (B.1.351) Spike RBD protein fused to Lucia luciferase

Catalog code: rbd-lucia-v3

<https://www.invivogen.com/b1351-rbd-lucia>

For research use only, not for diagnostic or therapeutic use

Version 23A10-MM

PRODUCT INFORMATION

Contents

- 50 µg of RBD-LuciaV3 (B.1.351) (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 Beta variant (B.1.351) Spike protein with a C-terminal Lucia luciferase reporter and histidine tag.

Sequence: GISAID EPI_ISL_745146 (codon optimized)

Origin: Beta Variant (B.1.351 lineage) | South African 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-LuciaV3 (B.1.351) 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

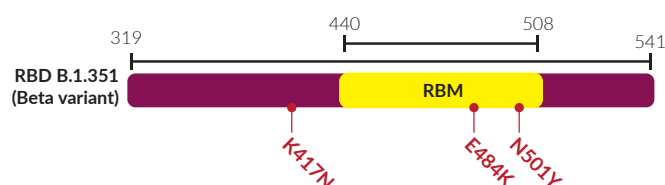
RBD-LuciaV3 (B.1.351) (~52 kDa) is a soluble fusion protein composed of the Receptor Binding Domain (RBD) from the SARS-CoV-2 Beta variant (B.1.351) Spike protein fused to a C-terminal Lucia luciferase reporter. RBD-LuciaV3 (B.1.351) has been generated by recombinant DNA technology, produced in CHO cells, 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 ACE2¹.

SARS-CoV-2 Spike RBD

RBD-LuciaV3 (B.1.351) contains the Spike RBD from the SARS-CoV-2 Beta variant (B.1.351), first reported in South Africa in October 2020. This variant is characterized by the presence of a number of mutations within the Spike RBD coding region, which are of concern².

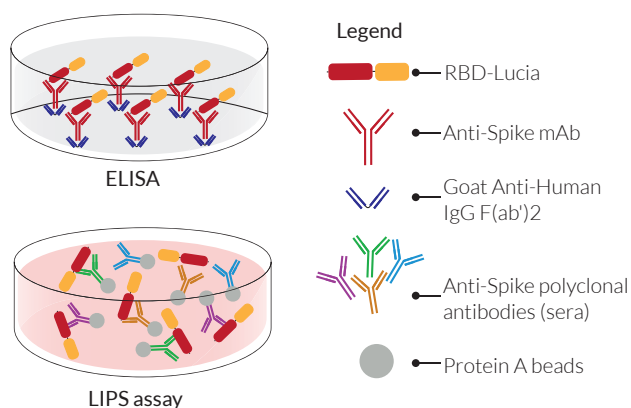
- K417N, E484K, N501Y



APPLICATIONS

RBD-LuciaV3 (B.1.351) 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 vaccinees³. 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. Garcia-Beltran, W.F. *et al.* 2021. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell*. doi:10.1016/j.cell.2021.03.013.
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.

TECHNICAL SUPPORT

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METHODS

RBD-LuciaV3 (B.1.351) 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-LuciaV3 (B.1.351) protein

1. Thaw an RBD-LuciaV3 (B.1.351) (100 µg/ml) aliquot
2. Prepare a 1 µg/ml working solution of RBD-LuciaV3 (B.1.351) 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 the prepared [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-LuciaV3 (B.1.351) protein

1. Thaw an RBD-LuciaV3 (B.1.351) (100 µg/ml) aliquot
2. Prepare a 10 µg/ml working solution of RBD-Lucia 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-hlgG1](#), 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-LuciaV3 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 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 centrifugation, 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-hlgG1	srbd-mab1
Anti-Spike-RBD-mIgG2a	srbd-mab10
Anti-CoV2RBD-cas-mIgG2a	srbd-c3-mab10
Anti-CoV2RBD-imd-mIgG2a	srbd-c4-mab10
Anti-CoV2RBD-bam-mIgG2a	srbd-c5-mab10
Anti-CoV2RBD-ete-mIgG2a	srbd-c6-mab10
Anti-βGal-hlgG1	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>

TECHNICAL SUPPORT

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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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