

RBD-LuciaV8 (B.1.617.2)

Soluble SARS-CoV-2 Delta variant (B.1.617.2) Spike RBD protein fused to Lucia luciferase

Catalog code: rbd-lucia-v8

<https://www.invivogen.com/b16172-rbd-lucia>

For research use only, not for diagnostic or therapeutic use

Version 25C04-MM

PRODUCT INFORMATION

Contents

- 50 µg of RBD-LuciaV8 (B.1.617.2) (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 Delta variant (B.1.617.2) Spike protein with a C-terminal Lucia luciferase reporter and histidine tag.

Sequence: GISAID EPI_ISL_2356230 (codon optimized)

Origin: Delta Variant (B.1.617.2 lineage) | Indian 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-LuciaV8 (B.1.617.2) 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

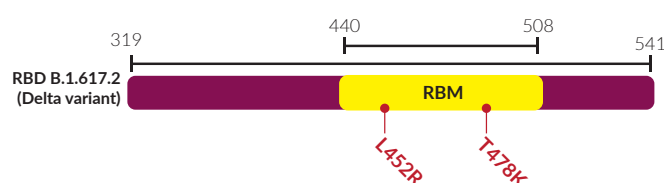
RBD-LuciaV8 (B.1.617.2) (~52 kDa) is a soluble fusion protein composed of the Receptor Binding Domain (RBD) from the SARS-CoV-2 Delta variant (B.1.617.2) Spike protein fused to a C-terminal Lucia luciferase reporter. RBD-LuciaV8 (B.1.617.2) has been generated by recombinant DNA technology, produced in 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-LuciaV8 (B.1.617.2) contains the Spike RBD from the SARS-CoV-2 Delta variant (B.1.617.2), first reported in India in October 2020. This variant is characterized by the presence of two key mutations within the Spike RBD coding region, which are of concern².

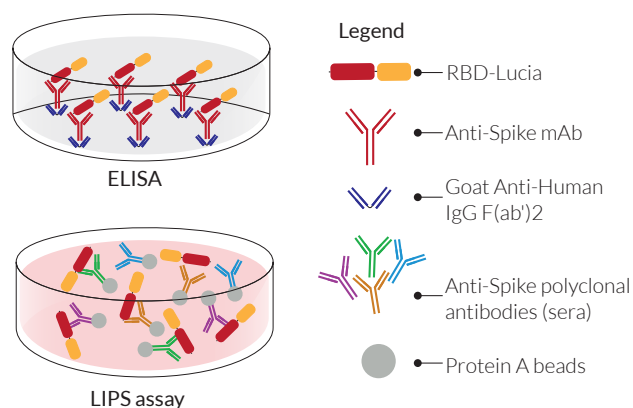
- L452R, T478K



APPLICATIONS

RBD-LuciaV8 (B.1.617.2) 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 other side 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. Davis, C. *et al.* 2021. Reduced neutralisation of the Delta (B.1.617.2) SARS-CoV-2 variant of concern following vaccination. *medRxiv* doi:10.1101/2021.06.23.21259327.
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

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com

METHODS

RBD-LuciaV8 (B.1.617.2) 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-LuciaV8 (B.1.617.2) protein

1. Thaw an RBD-LuciaV8 (B.1.617.2) (100 µg/ml) aliquot
2. Prepare a 1 µg/ml working solution of RBD-LuciaV8 (B.1.617.2) 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-mlgG2a](#) and [Anti-βGal-mlgG2a](#), 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-LuciaV8 (B.1.617.2) protein

1. Thaw an RBD-LuciaV8 (B.1.617.2) (100 µg/ml) aliquot
2. Prepare a 10 µg/ml working solution of RBD-LuciaV8 (B.1.617.2) 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-mlgG2a](#) 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. 6 µl of pre-equilibrated Protein A bead suspension
 - b. 10 µl of RBD-LuciaV8 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 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-mlgG2a	srbd-mab10
Anti-CoV2RBD-cas-mlgG2a	srbd-c3-mab10
Anti-CoV2RBD-imd-mlgG2a	srbd-c4-mab10
Anti-CoV2RBD-bam-mlgG2a	srbd-c5-mab10
Anti-CoV2RBD-ete-mlgG2a	srbd-c6-mab10
Anti-βGal-hlgG1	bgal-mab1
Anti-βGal-mlgG2a	bgal-mab10

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

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

