RBD-LuciaV7 (B.1.617.1)

Soluble SARS-CoV-2 Kappa variant (B.1.617.1) Spike RBD protein fused to Lucia luciferase

Catalog code: rbd-lucia-v7

https://www.invivogen.com/b16171-rbd-lucia

For research use only, not for diagnostic or therapeutic use

Version 23A11-MM

PRODUCT INFORMATION

Contents

- **50 µg of RBD-LuciaV7 (B.1.617.1)** (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 Kappa variant (B.1.617.1) Spike protein with a C-terminal Lucia luciferase reporter and histidine tag.
Sequence: GISAID EPI_ISL_1544071 (codon optimized)
Origin: Kappa Variant (B.1.617.1 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-LuciaV7 (B.1.617.1) 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.

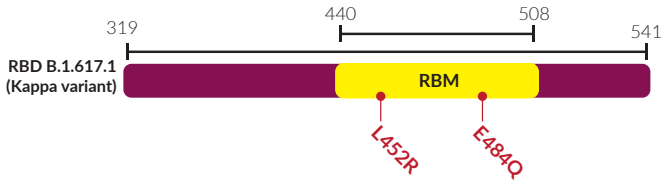
PRODUCT DESCRIPTION

RBD-LuciaV7 (B.1.617.1) (~52 kDa) is a soluble fusion protein composed of the Receptor Binding Domain (RBD) from the SARS-CoV-2 Kappa variant (B.1.617.1) Spike protein fused to a C-terminal Lucia luciferase reporter. RBD-LuciaV7 (B.1.617.1) 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-LuciaV7 (B.1.617.1) contains the Spike RBD from the SARS-CoV-2 Kappa variant (B.1.617.1), first reported in Inida in October 2020. This variant is characterized by the presence of two key mutations within the Spike RBD coding region, which are of concern[2].

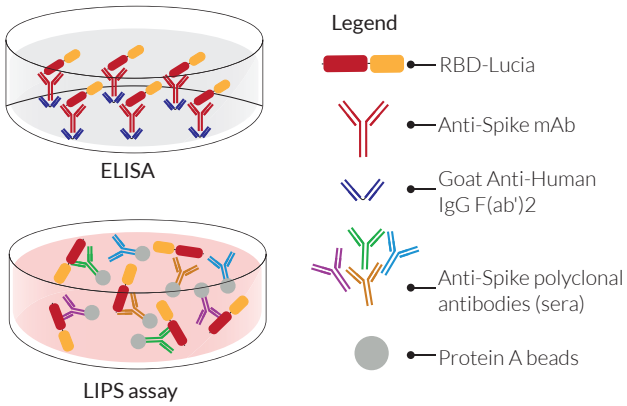
- **L452R, E484Q**



APPLICATIONS

RBD-LuciaV7 (B.1.617.1) 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. **Liu, C. *et al.* 2021.** Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell, doi:10.1016/j.cell.2021.06.020. 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-LuciaV7 (B.1.617.1) 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-LuciaV7 (B.1.617.1) protein

1. Thaw an RBD-V7 (B.1.617.1)(100 µg/ml) aliquot
2. Prepare a 1 µg/ml working solution of RBD-V7 (B.1.617.1) 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-LuciaV7 (B.1.617.1) protein

1. Thaw an RBD-LuciaV7 (B.1.617.1) (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-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-LuciaV7 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 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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