

# EK1C4

Pan-coronavirus fusion inhibitor  
Catalog code: inh-ek1c4, inh-ek1c4-1

<https://www.invivogen.com/ek1c4>

For research use only

Version 22B14-NJ

## PRODUCT INFORMATION

### Contents

EK1C4 is provided as a dried powder and is available in two pack sizes:

- 25 µg: inh-ek1c4
- 100 µg (4 x 25 µg): inh-ek1c4-1

### Storage and stability

- EK1C4 is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots and store at -20 °C. Resuspended product is stable for 6 months at -20 °C when properly stored. Avoid repeated freeze-thaw cycles.

### Quality control

- Purity: ≥95% (UHPLC)
- The inhibitory activity has been confirmed using in-house cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

## DESCRIPTION

EK1C4 is a lipopeptide that potently inhibits SARS-CoV-2 (and other coronaviruses) fusion with target cells<sup>1</sup>. It is derived from the EK1 peptide to which cholesterol has been covalently attached in the C-terminal with the help of a flexible polyethylene glycol (PEG) spacer<sup>1</sup>. EK1 was designed from the heptad repeat domain 2 (HR2) in the S2 subunit of the Spike (S) protein at the surface of human coronaviruses (HCoV)<sup>2</sup>. HR1 and HR2 trimers interact to form a coiled-coil, six-helix bundle (6-HB), which brings the viral and target cell membranes in close proximity for fusion.

EK1C4 binding to HR1 prevents the interaction with HR2. The cholesterol group improves the anti-viral activity of EK1C4, possibly through anchoring the inhibitor to the target membrane or binding to the hydrophobic groove on HR1 trimers<sup>1</sup>. EK1C4 has been described as the most potent HCoV fusion/entry inhibitor among EK1 and EK1-derived molecules in cellular assays using pseudotyped or live coronaviruses<sup>1</sup>. Moreover, intranasally applied EK1C4 protects mice against HCoV-OC43 infection<sup>1</sup>. This lipopeptide has thus the potential to be developed as a pan-coronavirus prophylactic or therapeutic, alone or in combination with neutralizing anti-Spike antibodies.

1. Xia, S. et al., 2020. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. *Cell Research*. 30(4):343-355. 2. Xia, S. et al., 2020. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cell Mol. Immunol.* 17:765-767.

## CHEMICAL PROPERTIES

CAS number: 2428532-99-2

Synonym: (N)EK1-GSGSG-PEG4-(Chol)

Formula: C<sub>250</sub>H<sub>405</sub>N<sub>51</sub>O<sub>78</sub>S<sub>2</sub>

Molecular weight: 5433.9 g/mol

Solubility: 5 mg/ml (0.92 mM) in DMSO

## METHODS

Preparation of stock solution (1 mg/ml)

1. Add 25 µl DMSO to 25 µg EK1C4 vial.
2. Vortex until completely resuspended.
3. Prepare aliquots of EK1C4 and store at -20 °C.
4. Once EK1C4 is resuspended, further dilutions can be prepared using sterile aqueous buffers.

Working concentration range: 0.5 nM - 2 µM for cell culture assays using the Wuhan (original) Spike.

## PROTOCOL

Below is a protocol to assess the inhibition of Spike-ACE2-dependent cell fusion. This assay relies on the transfer of the adaptor molecule, MyD88, from a 'donor cell line' to an 'acceptor cell line' expressing an NF-κB-SEAP inducible reporter gene.

- 'Donor cells' are transiently or stably transfected with an optimized expression plasmid featuring one of the Spike variants.
- 'Acceptor cells' stably express the Spike receptors, ACE2 & TMPRSS2. The neutralizing ability of EK1C4 is determined by measuring the reduction of SEAP production. For more information, visit: <https://www.invivogen.com/cell-fusion>.

### Generation of 293-hMyD88-Spike 'donor cells'

1. Wash 293-hMyD88 cells with PBS and detach cells with trypsin.
2. Centrifuge cells at 300 x g (RCF) for 5 min.
3. Remove supernatant and resuspend cells at 0.3 x 10<sup>6</sup> cells/ml in fresh, pre-warmed growth medium
4. Add 3 ml of cell suspension (~1 x 10<sup>6</sup> cells) per well of a 6-well plate.
5. Combine 1.5 µg pUNO1-Spike with 150 µL LyoVec™ transfection reagent and incubate at room temperature for 30 mins.  
*Note:* InvivoGen offers a comprehensive collection of expression plasmids encoding various Spike variants (e.g. Wuhan (original), Delta, Kappa, etc...). For more information: <https://www.invivogen.com/sars2-spike-vectors>
6. Add 150 µl of prepared complex to the cell-containing wells.
7. Incubate the plate for 24h or 48h at 37°C, 5% CO<sub>2</sub>.

## TECHNICAL SUPPORT

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8. Wash pre-prepared transfected cells (293-hMyD88-Spike) with PBS and detach in PBS by tapping the plate.
9. Centrifuge cells at 300 x g (RCF) for 5 min.
10. Remove supernatant and prepare a suspension at  $4 \times 10^5$  cells/ml in fresh, pre-warmed test medium.

#### Preparation of HEK-Blue™ hACE2-TMPRSS2 'acceptor cells'

1. Gently rinse HEK-Blue™ hACE2-TMPRSS2 cells twice with pre-warmed PBS and detach the cells in PBS by tapping the flask. Dissociate cell clumps by gently pipetting up and down.  
*Note: Do not use trypsin to detach HEK-Blue™ hACE2-TMPRSS2 cells.*
2. Centrifuge cells at 300 x g (RCF) for 5 min.
3. Remove supernatant and prepare a suspension at  $2 \times 10^5$  cells/ml in fresh, pre-warmed test medium.

#### Co-culture of 'donor' and 'acceptor' cells

1. Prepare a 1:3 serial dilution of EK1C4 using test medium in a flat-bottom 96-well plate. Start with a final concentration of 10 µg/ml/well and final volume of 100 µl per well.
2. Add 50 µl of prepared 293-hMyD88-Spike cell suspension (20,000 cells) per well.
6. Add 50 µl of prepared HEK-Blue™ hACE2-TMPRSS2 cell suspension (10,000 cells) per well.
7. Incubate the plate overnight at 37°C, 5% CO<sub>2</sub>.

#### Measuring cell fusion

1. Prepare QUANTI-Blue™ Solution as per the product data sheet.
2. Dispense 180 µl of QUANTI-Blue™ Solution per well of a new flat-bottom 96-well plate.
3. Add 20 µl of cell fusion supernatant per well.
4. Incubate the plate at 37°C for 30 min to 1 hour.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

## RELATED PRODUCTS

Product	Cat. Code
293-hMyD88 cells	293-hmyd
HEK-Blue™ hACE2-TMPRSS2 cells	hkb-hace2tpsa
QUANTI-Blue™ Solution	rep-qbs

InvivoGen also offers a collection of SARS-CoV-2 Spike Expression Plasmids. For more information, please visit: <https://www.invivogen.com/sars2-spike-vectors>.

InvivoGen also offers a collection of SARS-CoV-2 Spike Pseudotyping Vectors. For more information, please visit: <https://www.invivogen.com/spike-pseudotyping-vectors>.

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