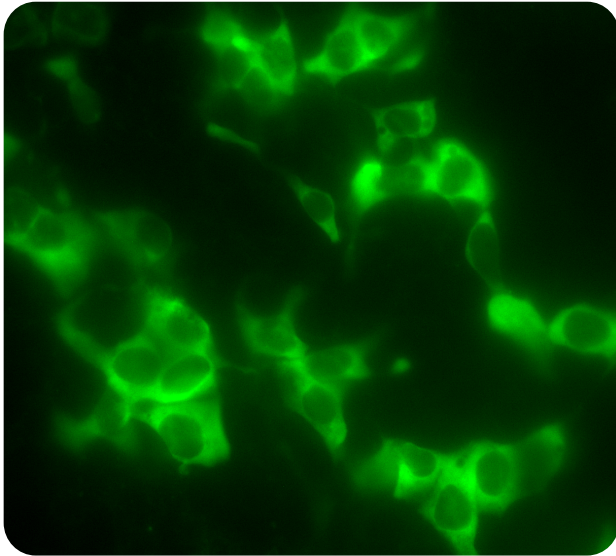




ExpressCells

Nucleocapsid [SARS-CoV-2] Overexpression cell line (HEK 293T)

Catalog no: EXP-014-HET



Product summary

In this cell line, N, the gene coding the SARS-CoV-2 nucleocapsid protein, has been codon optimized and knocked in to intron 1, *CLYBL* with the EF1 α promoter and C-terminal mClover3, FLAG-, and His-tags. Expression of the nucleocapsid protein has been validated by fluorescence microscopy and western blotting.

Cell type:	HEK 293T
Expression model:	Overexpression; safe-harbor knock-in with promoter
Gene symbol / ORF name / NCBI gene ID:	N / 9a / 43740575
Labeling tags:	mClover3, 3X FLAG, 6xHis
Genomic sequence:	NC_045512.2 (28274..29533)
Knock-in site:	Intron 1, <i>CLYBL</i>
Expressed protein / amino acid residues:	Nucleocapsid protein [SARS-CoV-2] / 419
Antibiotic resistance:	Puromycin
Validation:	PCR, DNA sequencing at the genomic insertion site, and western blot
Population type:	Heterozygous

Protein summaries from NCBI database and UniProt

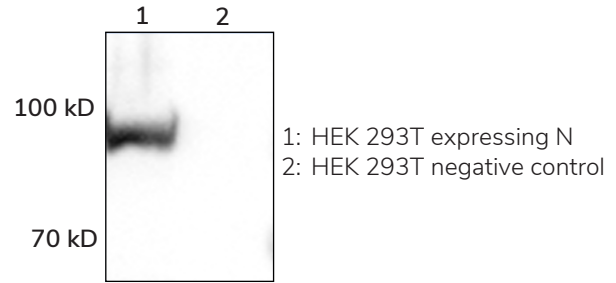
The nucleocapsid protein, N, is one of four structural proteins in SARS-CoV-2, which also include S (spike), E (envelope), and M (membrane). It binds to and protects viral RNA and is involved in packaging positive-strand viral RNA into a helical ribonucleocapsid.

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CUSTOM CELL LINE SERVICES AVAILABLE UP TO 3 KNOCK-INS IN A SINGLE CELL LINE

Like the nucleocapsid protein of SARS-CoV, this protein might play an important role in enhancing the efficiency of subgenomic viral RNA transcription as well as viral replication, and could modulate transforming growth factor-beta signaling by binding host SMAD3.

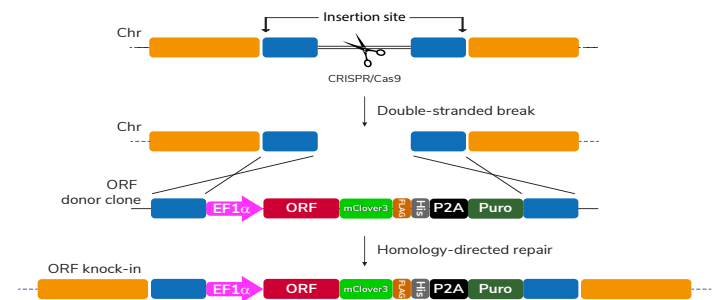
The N protein has been proposed as a target for antiviral drugs.



Immunoblot of SARS-CoV-2 N protein tagged with mClover-3, 3X FLAG, 6xHis using 10 μ g whole-cell lysate and anti-FLAG tag antibody.

ExpressCells' FAST-HDR knock-in technology

ExpressCells uses CRISPR and FAST-HDR vector technology to rapidly knock foreign genes into mammalian DNA, whether to tag endogenously expressed genes or to create over-expression models.



Handling

Culture medium: Dulbecco's Modified Eagle Medium (DMEM), high glucose supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin to prevent bacterial contamination.

Thawing: Transfer the frozen tube to a 37 $^{\circ}$ C water bath and let contents thaw. Transfer tube contents to 10 mL of prewarmed medium in a biosafety hood and centrifuge at 200 \times g for 5 min. Resuspend the pellet in 5 mL of medium and transfer to a mammalian cell culture flask.

Safety: Biosafety level 2.

References

- Gene [database online]. Washington DC: NCBI; 2020. <https://www.ncbi.nlm.nih.gov/gene/?term=sars-cov-2+nucleocapsid>. Accessed July 6, 2020.
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- Stert S, Reichelt M, Spiegel M, Kuri T, et al The intracellular sites of early replication and budding of SARS-coronavirus. *Virology*. 2007;361:304-15.
- Zhao X, Nicholls JM, Chen Y-G Severe acute respiratory syndrome-associated coronavirus nucleocapsid protein interacts with SMAD3 and modulates transforming growth factor-beta signaling. *J Biol Chem*. 2008;283:3272-80.
- Perez-Leal O, Nixon-Abell J, Barrero CA, Gordon J, Rico MC. A versatile vector system for the fast generation of knock-in cell lines with CRISPR [preprint published online February 6 2020]. *bioRxiv*. doi: 10.1101/2020.02.06.927384.

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