

pDUO2-hACE2-TMPRSS2a

Expression vector containing human ACE2 and TMPRSS2a open reading frames

Catalog code: pduo2-hace2tpsa

<https://www.invivogen.com/human-ace2-tmprss2a-expression-vector>

For research use only

Version 20F02-NJ

PRODUCT INFORMATION

Contents

- 20 µg of pDUO2-hACE2-TMPRSS2a provided as lyophilized DNA
- 1 ml of Hygromycin B Gold at 100 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C and is stable at least for 1 year.
- Store Hygromycin B Gold at 4°C or -20°C. The expiry date is specified on the product label.

Quality control

- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.
- Plasmid construct is confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.

GENERAL PRODUCT USE

- **pDUO2** expression vectors are designed to co-express two distinct gene coding sequences using distinct and equally potent mammalian expression cassettes.

- **Stable gene expression in mammalian cells.** pDUO2 plasmids can be used directly in transfection experiments both *in vitro* and *in vivo*. pDUO2 plasmids contain the hygromycin-resistance gene (*hph*). In bacteria, *hph* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *hph* is transcribed from the human FerH composite promoter as a polycistronic mRNA and translated via the FMDV IRES. This allows the amplification of the plasmid in *E. coli*, as well as the selection of stable clones in mammalian cells using the same selective antibiotic. pDUO2 allows high and similar levels of expression and secretion of the two gene products from each cassette.

METHODS

• Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water. Store resuspended plasmid at -20°C.

• Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

• Hygromycin B Gold usage

Hygromycin B Gold should be used for *E. coli* at 50-100 µg/ml in liquid or solid media, and at 50-500 µg/ml to select hygromycin-resistant mammalian cells.

PLASMID FEATURES

- **human ACE2** **ORF size:** 2418 bp
- **human TMPRSS2a** **ORF size:** 1590 bp

Human ACE2 (angiotensin I-converting enzyme-2) is a type I surface transmembrane protein expressed in arteries, heart, kidneys, and epithelia of the lung and small intestine^{1,2}. ACE2 belongs to the angiotensin-converting enzyme family of dipeptidyl carboxydipeptidases^{2,3}.

Human TMPRSS2a (transmembrane protease serine 2, isoform 1) is a multimeric protein containing four domains, among which a type II transmembrane domain and a serine protease domain. It is widely expressed in epithelial tissues, including prostate, pancreas, liver, kidney, lung, colon, and small intestine⁴. TMPRSS2 is capable of autoactivation, and its protease domain is thought to be secreted upon autocleavage⁵.

ACE2 and TMPRSS2 play a critical role in the human pathogenesis of the coronavirus disease-19 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Indeed, hACE2 has been described as a receptor for the Spike (S) protein of the SARS-CoV and SARS-CoV-2 coronaviruses. The binding of the S protein to ACE2 with the concerted action of host proteases, including TMPRSS2, facilitates the viral entry into target cells⁶⁻⁸. While the exact timing and location for these processes to take place remain to be determined, it has been proposed that the S protein is cleaved into two subunits (S1 and S2) by extracellular proteases, including furin⁹. S1 binds to ACE2 and S2 is further cleaved and activated by the host surface-associated transmembrane protease serine 2 (TMPRSS2)^{7,9}. Together these actions result in host-viral membrane fusion and the viral RNA genome is released into the host cell cytoplasm.

- **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein¹⁰. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.

- **SV40 enhancer:** It is comprised of a 72-base-pair repeat and it allows the enhancement of gene expression in a wide range of hosts. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids¹¹.

- **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is several fold more active than the SV40 enhancer¹².

TECHNICAL SUPPORT

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- **SV40 pAn:** The Simian Virus 40 late polyadenylation (pAn) signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA¹³.
- **pMB1 ori:** A minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression¹⁴.
- **EM7:** A bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Hph (hygromycin resistance gene):** It confers resistance to Hygromycin B both in *E. coli* and mammalian cells. In bacteria, *hph* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *hph* is transcribed from the human FerH composite promoter as a polycistronic mRNA and translated via the FMDV IRES.
- **EF1 pAn:** A strong polyadenylation (pAn) signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

RELATED PRODUCTS

Product	Description	Cat. Code
Hygromycin B Gold	Selection antibiotic	ant-hg-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
pUNO1His-SARS2-S	Production vector	p1his-cov2-s
pUNO1Fc-SARS2-S	Production vector	p1fc-cov2-s
pUNO1His-SARS2-S1	Production vector	p1his-cov2-s1
pUNO1Fc-SARS2-S1	Production vector	p1fc-cov2-s1
pUNO1His-SARS2-RBD	Production vector	p1his-cov2-rbd
pUNO1Fc-SARS2-RBD	Production vector	p1fc-cov2-rbd

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