abcam

Product datasheet

Human TMPRSS2 knockout LNCaP cell line ab273745

画像数 4

製品の概要

製品名	Human TMPRSS2 knockout LNCaP cell line
Parental Cell Line	LNCaP
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 79 bp deletion in exon 3
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
アプリケーション	**適用あり:** Sandwich ELISA, ICC
Biosafety level	1
特記事項	**Recommended control:** Human wild-type LNCaP cell line (**ab275470**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** RPMI + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $1x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. Cells grow in "islands". A guide seeding density of $1x10^4$ cells/cm^2 is recommended. Cells should be passaged when they have achieved $2x10^5$ cm^2.

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We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Prostate
Cell type	epithelial
Disease	Carcinoma
Gender	Male
Mycoplasma free	Yes
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

組織特異性	Expressed strongly in small intestine. Also expressed in prostate, colon, stomach and salivary gland.
配列類似性	Belongs to the peptidase S1 family. Contains 1 LDL-receptor class A domain. Contains 1 peptidase S1 domain. Contains 1 SRCR domain.
細胞内局在	Cell membrane and Secreted. Activated by cleavage and secreted.

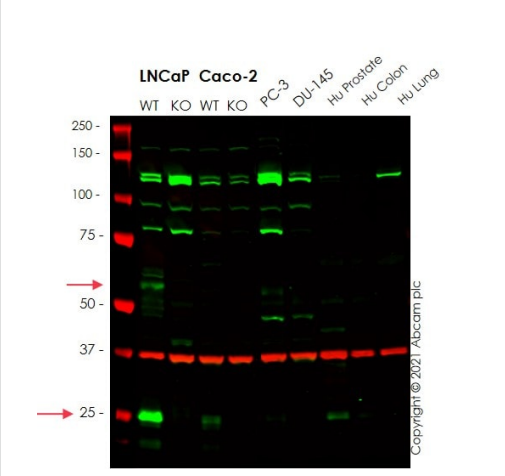
アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab273745の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Sandwich ELISA		Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.

画像



Western blot - Human TMPRSS2 knockout LNCaP cell line (ab273745)

All lanes : Anti-TMPRSS2 antibody [EPR3862] (**ab109131**) at 1/2000 dilution

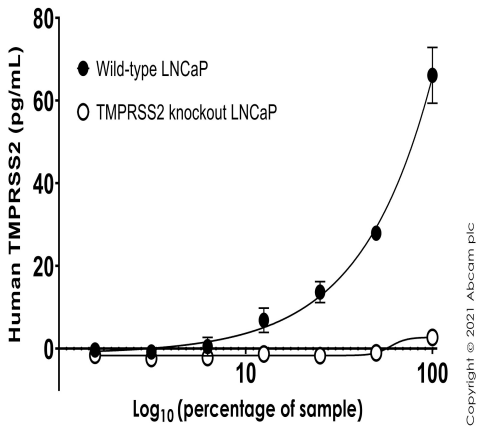
Lane 1 : Wild-type LNCaP cell lysate
Lane 2 : TMPRSS2 knockout LNCaP cell lysate
Lane 3 : Wild-type Caco-2 cell lysate
Lane 4 : TMPRSS2 knockout Caco-2 cell lysate
Lane 5 : PC-3 cell lysate
Lane 6 : DU 145 cell lysate
Lane 7 : Human Prostate cell lysate
Lane 8 : Human Colon cell lysate
Lane 9 : Human Lung cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

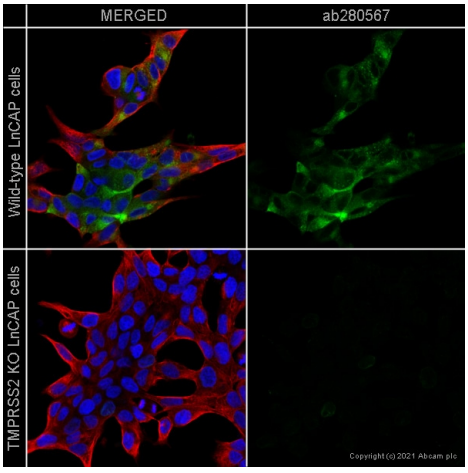
Observed band size: 55,25 kDa

False colour image of Western blot: Anti-TMPRSS2 antibody [EPR3862] staining at 1/2000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab109131** was shown to bind specifically to TMPRSS2. A band was observed at 55, 25 kDa in wild-type LNCaP and at 25 kDa in Caco-2 cell lysates with no signal observed at this size in TMPRSS2 knockout LNCaP cell line ab273745 (knockout LNCaP cell lysate **ab275499**) and TMPRSS2 knockout Caco-2 cell line **ab273737** (knockout Caco-2 cell lysate **ab277340**). To generate this image, wild-type and TMPRSS2 knockout cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Sandwich ELISA - Human TMPRSS2 knockout LNCaP cell line (ab273745)

Human TMPRSS2 concentration was interpolated from the TMPRSS2 standard curve. Supernatants from cell culture samples were serially diluted and assessed by the Human TMPRSS2 ELISA Kit (**ab283552**). Wild-type and TMPRSS2 knockout LNCaP (ab273745) cells were assessed in duplicate (n=2). Data are represented as the mean and error bars represent standard deviation.



Immunocytochemistry - Human TMPRSS2 knockout LNCaP cell line (ab273745)

ab280567 staining TMPRSS2 in wild-type LnCAP cells (top panel) and TMPRSS2 knockout LnCAP cells (ab273745) (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab280567** at 1µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

Sanger Sequencing - Human TMPRSS2 knockout LNCaP cell line (ab273745)

79 bp deletion in exon 3

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