



# HEK293-FAP

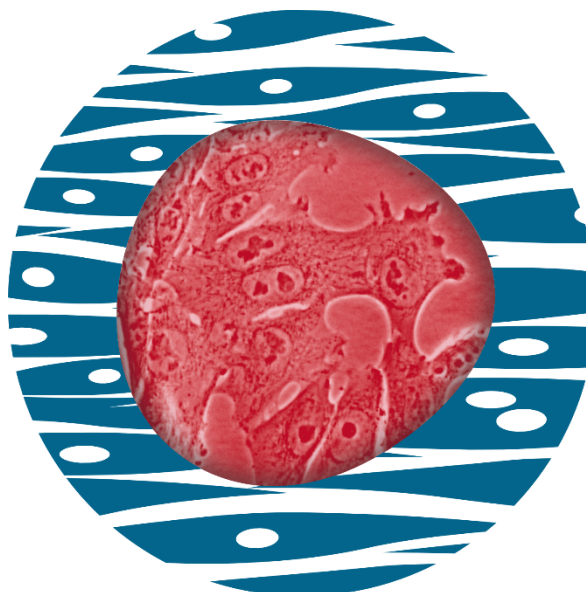
Cat.-No.: INS-SF-1001

**FAP Expressing Stable Recombinant**

**HEK293 Cell Line**

## Product Sheet

→ At a glance		
<b>BSL</b> <i>Level 1</i>	<b>Coating</b> <i>Collagen Coating (INS-SU-1017) for initial recovery</i>	<b>Growth</b> <i>Adherent</i>
<b>Storage</b> <i>&lt;2d: -80°C &gt;2d: liquid Nitrogen</i>	<b>Medium</b> <i>HEK293 Growth Medium A (INS-ME-1041)</i>	<b>Expression Level</b> <i>High (123000 molecules/cell)</i>





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## → Intended Use and Licensing

*This product is intended for in vitro laboratory research use only. It is not intended for any animal or human therapeutic or diagnostic use.*

If you have purchased this cell line for **academic, non-profit research**, the use of this cell line is governed by the **inscreenex Limited Research Use License (LRUL)**. Please refer to the LRUL for the full terms and conditions, and relevant use limitations. If you have purchased this cell line for **commercial, for-profit research**, the use of this cell line is governed by the **inscreenex Limited Commercial Use License (LCUL)**. Please refer to the LCUL for the full terms and conditions, and relevant use limitations. If you wish to use the cell line for commercial purposes that fall outside the permitted use in the LCUL please contact [licensing@inscreenex.com](mailto:licensing@inscreenex.com).



## → Background Information

*HEK293-FAP is a recombinant HEK293 cell line expressing full length human FAP (human Fibroblast Activation Protein).*

**Catalog number:** INS-SF-1001

**Target:** human FAP

**Target aliases:** SEPR, Seprase, DPPIV

**Target expression level(s):** High (approx. 123000 molecules/cell)

**Biosafety level (BSL):** Level 1

**Cell Background:** HEK293 (*Homo sapiens*, human)

**Growth properties:** adherent

### Target Background

FAP, or Fibroblast Activation Protein, is an integral serine protease of the post-proline cleaving enzyme family, closely associated with dipeptidyl peptidase-IV. Its primary role is in the remodeling of the extracellular matrix, which becomes particularly relevant in wound healing, tissue repair, and fibrosis. Moreover, FAP exhibits a unique expression pattern, being highly upregulated in the stroma of many epithelial cancers, which includes its presence in cancer-associated fibroblasts. This expression profile has established FAP as a potential biomarker and therapeutic target in oncology.

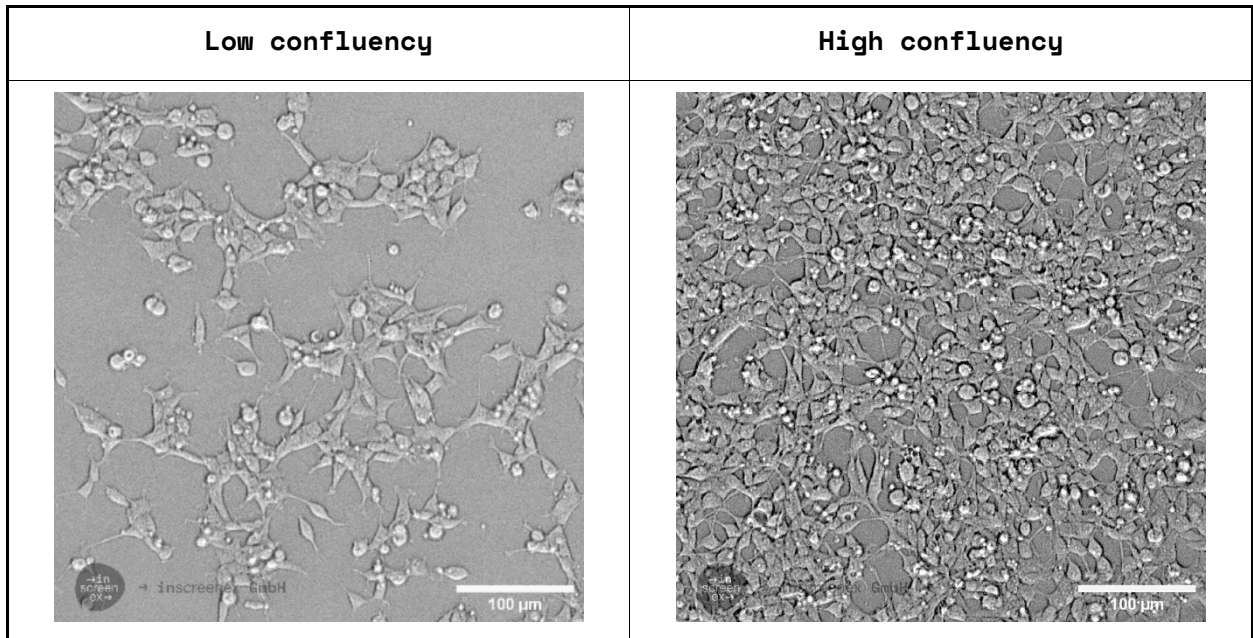
<b>Note</b>	<i>All target sequences undergo codon optimization and other sequence modifications on DNA level to improve recombinant expression and the nucleotide sequence of the recombinant protein therefore differs from database reference sequences. For details refer to section <a href="#">Target Sequence</a> on page <a href="#">11</a>.</i>
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### Cell Line Generation

This cell line was generated using our inscreenex landing pad cell lines. These cells contain a recombination site and a selectable marker at a pre-validated genomic locus. Using a matching recombinase and specifically designed expression setups, the DNA payload, i.e. the target, is then specifically inserted into that locus, allowing for reproducible integration at well-defined sites in the genome. This significantly reduces the effort and timelines to isolate a stable clonal population. Expression of the target was then analyzed using flow cytometry and target-specific antibodies.

## → Morphology

*adherent; epithelial-like; grows in monolayer*

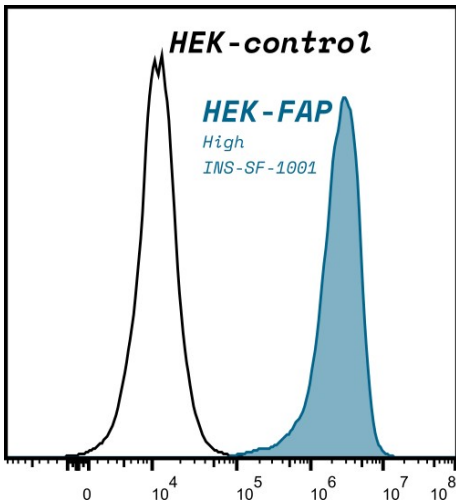


## → Cell Characterization

### Target Expression Level

*Target expression was analyzed using a target-specific antibody and the indicated staining protocol.*

Materials	Protocol
<ul style="list-style-type: none"> <li>– PBS/EDTA solution</li> <li>– 2% FBS/FCS in PBS (FACS Buffer)</li> <li>– Primary antibody: Human FAP Antibody (R&amp;D systems, #MAB3715)</li> <li>– Secondary antibody: PE-Goat anti-mouse IgG (Biolegend, #405307)</li> </ul>	<p><b>Wash Protocol:</b> Add FACS Buffer, resuspend cells gently, then centrifuge at 300×g for 5min.</p> <ol style="list-style-type: none"> <li>1) Prepare detection reagents in FACS buffer.</li> <li>2) Aspirate medium from cells.</li> <li>3) Add PBS/EDTA solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach.</li> <li>4) Wash cells 1×.</li> </ol>

Expression Profile	
 <p><b>HEK-control</b></p> <p><b>HEK-FAP</b> High INS-SF-1001</p> <p><b>HEK-control:</b> HEK293 landing pad cell line transfected with a scrambled sequence.</p> <p><b>HEK-FAP:</b> HEK293 landing pad cell line transfected with the target.</p> <p>→ Both samples were stained with antibodies and analyzed according to the Protocol section.</p>	<ol style="list-style-type: none"> <li>5) Add primary antibody in FACS buffer, resuspend cells gently.</li> <li>6) Incubate at ambient temperature for 20-30min.</li> <li>7) Wash cells 2x.</li> <li>8) Add secondary antibody in FACS buffer, resuspend cells gently.</li> <li>9) Incubate on ice for 15-20min.</li> <li>10) Wash cells 2x.</li> <li>11) Resuspend in 100-200µl FACS Buffer.</li> <li>12) Analyze cells using a flow cytometer.</li> </ol>

## Receptor Density

Receptor density was estimated using the BD Quantibrite™ System.

Materials	Protocol
<ul style="list-style-type: none"> <li>– BD Quantibrite™ PE Phycoerythrin Fluorescence Quantitation Kit (#340495)</li> <li>– 2% FBS/FCS in PBS (FACS Buffer)</li> </ul>	<p>For a detailed protocol refer to the manufacturer's protocol (<a href="#">Link</a>).</p> <ol style="list-style-type: none"> <li>1) Reconstitute Beads in FACS Buffer.</li> <li>2) Measure Beads in the same run as cells using the same flow cytometer settings.</li> <li>3) Convert Signal to PE molecules/cell according to manufacturer's instructions.</li> </ol>
<p><b>Expression Level</b></p>	
<p>High (approx. 123000 molecules/cell)</p>	



## → Quality Control

Basic information on quality control can be found below. For more details, request a Certificate of Analysis (CoA) by emailing [info@inscreenex.com](mailto:info@inscreenex.com) and stating your Lot number.

**Cell number:** >0.5Mio viable cells (see info on vial label for exact cell number)

**Viability:** >75% post-thaw viability

**Sterility:** no contamination detected

**Mycoplasma:** no contamination detected

**Human pathogens:** Host cell line negative for HIV-1/2, HBV, HCV

## → Related Products

Products that are related to the HEK293-FAP cell line and are either required or recommended for a successful cell culture.

Required	Recommended
<b>Medium:</b> HEK293 Growth Medium A (INS-ME-1041) <b>Coating solution:</b> Collagen Coating (INS-SU-1017) for best attachment after thawing.	<b>Cryopreservation:</b> Cell Freezing Medium (INS-SU-1027)

## → Upon Arrival

Cells are routinely shipped on dry ice. Check all containers for leakage and breakage. Check if cells arrived frozen.

If, immediately upon arrival...	...Contact us:
<ul style="list-style-type: none"><li>– the vial appears damaged,</li><li>– the dry ice level in the shipping container appears low,</li><li>– the cells appear thawed, or</li><li>– you have any other concerns regarding the quality of the cells</li></ul>	<ol style="list-style-type: none"><li>1) take photos of the vial and/or the shipping container,</li><li>2) contact us by email or telephone (see <a href="#">General Inquiries</a> on page 2).</li></ol>
<b>If everything looks good, either seed the cryopreserved cells immediately, or store them:</b>	
<ul style="list-style-type: none"><li>– at -80°C for periods of up to 2 days, or</li><li>– below -130°C in liquid nitrogen vapor, for long term storage.</li></ul>	



## → Coating Protocol

Our Collagen Coating Solution is a sterile, ready-to-use solution ideal for coating cell culture surfaces.

<b>Note</b>	<p><i>For best attachment and viability after thawing the cells, we recommend using Collagen-coated flasks or plates for the initial seeding after cryo-recovery.</i></p> <p><i>Collagen coating is not required for subsequent routine culture of the cells.</i></p>
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Required materials	Protocol																
<ul style="list-style-type: none"> <li>– Cell culture plastic (flasks, dishes, plates)</li> <li>– Sterile PBS</li> <li>– Collagen Coating (INS-SU-1017)</li> </ul>	<ol style="list-style-type: none"> <li>1) Cover the cell culture vessel with Collagen Coating (see table to the left for recommended volumes). Rock back and forth to cover the entire surface if necessary.</li> <li>2) Incubate for at least 2h at 37°C in the incubator or overnight at 2–8°C.</li> <li>3) Aspirate Collagen Coating.</li> <li>4) Wash once with at least 4 volumes of sterile PBS.</li> <li>5) Aspirate PBS.</li> <li>6) Use immediately or store coated cell culture plastic according to instructions provided to the left.</li> </ol>																
<p><b>Storage</b></p> <ul style="list-style-type: none"> <li>– Store Collagen Coating at 2–8°C, if not indicated otherwise on the product label.</li> <li>– Store coated cell culture plastic sealed at 2–8°C for up to 7 days.</li> </ul>																	
<p><b>Recommended volumes</b></p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left;">Flask/Plate</th> <th style="text-align: left;">Coating Volume</th> </tr> </thead> <tbody> <tr><td>T75</td><td>3ml</td></tr> <tr><td>T25</td><td>1ml</td></tr> <tr><td>6-well</td><td>0.7ml</td></tr> <tr><td>12-well</td><td>0.25ml</td></tr> <tr><td>24-well</td><td>0.1ml</td></tr> <tr><td>48-well</td><td>75µl</td></tr> <tr><td>96-well</td><td>50µl</td></tr> </tbody> </table>		Flask/Plate	Coating Volume	T75	3ml	T25	1ml	6-well	0.7ml	12-well	0.25ml	24-well	0.1ml	48-well	75µl	96-well	50µl
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## → Medium Information

<b>Note</b>	<i>We provide a ready-to-use HEK293 Growth Medium A (INS-ME-1041) and Cell Freezing Medium (INS-SU-1027) for the culture and cryopreservation of HEK293-FAP cells.</i>
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### Ready-to-use HEK293 Growth Medium A

<b>Selection antibiotic</b>	<b>Anti-contamination antibiotics</b>
Our HEK293 Growth Medium A is shipped ready to use and already contains the G418 selection antibiotic (1mg/ml final) to guarantee stable long-term expression of the target.	Our HEK293 Growth Medium A does not contain prophylactic antibiotics for prevention of contamination. If you wish to use antibiotics, any standard, cell culture grade antibiotics can be added to the medium.

**Storage:** Store HEK293 Growth Medium A at 4 to 8°C.

**Stability:** See Expiry Date on bottle label.

## → Thaw Cryopreserved Cells

*Do not thaw the cells until the recommended medium and coated flasks are on hand. For initial recovery (after delivery of the cells), we recommend thawing the cells on a T25 flask and not exceeding a split ratio of 1:2 to 1:3 for the first split after thawing.*

<b>Note</b>	<i>For best attachment and viability after thawing the cells, we recommend using Collagen-coated flasks or plates for the initial seeding after cryo-recovery.</i>  <i>Collagen coating is not required for subsequent routine culture of the cells.</i>
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<b>Required materials</b>	<b>Protocol</b>
<ul style="list-style-type: none"> <li>– Coated cell culture vessel (see section <a href="#">Coating Protocol</a> on page <a href="#">7</a>)</li> <li>– HEK293 Growth Medium A (INS-ME-1041) pre-warmed to 37°C</li> <li>– 15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")</li> </ul>	<ol style="list-style-type: none"> <li>1) Add 4ml pre-warmed medium to a 15ml tube.</li> <li>2) Quickly thaw the cryovial at 37°C in a water bath until only a few ice crystals are visible. Disinfect vial briefly by spraying with 70% Ethanol.</li> <li>3) Transfer thawed cell suspension to the 15ml tube containing 4ml medium. Avoid excessively pipetting up and down.</li> <li>4) Centrifuge cells at 300×g for 5min.</li> <li>5) Aspirate supernatant.</li> <li>6) Gently resuspend the cell pellet in complete Medium. Use a volume appropriate for the cell culture vessel.</li> <li>7) Transfer cells in coated cell culture vessel and place in the incubator (37°C, 5% CO<sub>2</sub>).</li> <li>8) Change the medium after 2 days.</li> </ol>

## → Freeze Cells for Cryopreservation

*Cell should be grown to 90% confluence before cryopreservation. Avoid full confluence before cryopreservation.*

<b>Required materials</b>	<b>Protocol</b>
<ul style="list-style-type: none"> <li>– Cell Freezing Medium (INS-SU-1027)</li> <li>– PBS</li> <li>– Trypsin/EDTA solution (TE)</li> <li>– 2% FBS in PBS</li> <li>– 15ml tube</li> <li>– Cryovial(s)</li> <li>– Freezing container ("Mr. Frosty" or similar)</li> <li>– 15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")</li> </ul>	<ol style="list-style-type: none"> <li>1) Aspirate medium, wash with PBS and aspirate PBS.</li> <li>2) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach.</li> <li>3) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.</li> <li>4) Resuspend cells in 2% FBS in PBS and transfer to a 15ml conical bottom tube.</li> <li>5) Centrifuge cells at 300×g for 5min.</li> <li>6) Aspirate supernatant and gently resuspend cell pellet in Freezing medium (approx. 1Mio. cells/ml).</li> <li>7) Transfer cell suspension into cryovial(s) and place them into a freezing container ("Mr.Frosty" or similar).</li> <li>8) Place the freezing container at -80°C for 16-24h.</li> <li>9) Transfer cryovials to liquid nitrogen vapor for long-term-storage.</li> </ol>

## → Routine Adherent Cell Culture

*Work in a sterile environment and follow Good Cell and Tissue Culture Practice.*



**Temperature:** 37°C

**Environment:** 5% CO<sub>2</sub> (v/v), humidified atmosphere

**Split ratio:** 1:2 for initial split after thawing, 1:5 to 1:10 for routine culture

**Confluence:** split at 70–90% confluence

**Medium change:** every 2–3 days

<b>Required materials</b>			<b>Protocol</b>
<ul style="list-style-type: none"> <li>– HEK293 Growth Medium A (INS-ME-1041)</li> <li>– PBS</li> <li>– Trypsin/EDTA solution (TE)</li> </ul>			
<b>Recommended volumes</b>			
<b>Flask or Plate</b>	<b>Medium or PBS</b>	<b>TE solution</b>	
T75	8–10ml	3ml	
T25	4–5ml	1ml	
6-well	1.5–3ml	0.7ml	
12-well	1–2ml	0.25ml	
24-well	0.5–1ml	0.1ml	
48-well	0.2–0.4ml	75µl	
96-well	0.1–0.2ml	50µl	

- 1) Aspirate medium.
- 2) Wash with PBS and aspirate PBS.
- 3) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach.
- 4) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.
- 5) Resuspend cells in complete Medium thereby inactivating the Trypsin/EDTA (TE) solution.
- 6) Transfer an aliquot of the cell suspension to a new cell culture vessel containing fresh complete Medium.
- 7) Place into incubator.

