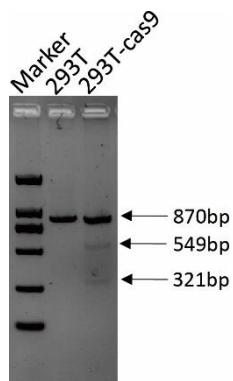


Cas9-expressing 293T Cell Line-BSD-Mcherry

Catalog #	EX0027567302
Description	EdiGene's Cas9-expressing 293T cell line-BSD-Mcherry has been developed by the integration of the CMV plasmid, which contains Cas9 genes and 2A-linked mcherry gene, as well as linked SV40 driven blasticidin resistant gene. The cell line is blasticidin resistant and has a high level constant expression of Cas9.
Plasmid Map	
Parental cell line	293T
Cell Type	Epithelial
Adherent/Suspension	Adherent
Organism	Homo sapiens
Amount	1×10 ⁶ cells/tube, 1mL
Complete Medium	DMEM, 10% FBS
Handing Procedure	<p>Upon arrival, it should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C will result in loss of viability.</p> <ol style="list-style-type: none"> 1. Thaw the vial in 37°C water bath approximately 1-2 minutes. 2. Transfer the cell suspension to a 15mL conical tube with pre-warmed 5mL DMEM+10% FBS, spin 125×g for approximately 5 minutes at room temperature. 3. Resuspend the cell pellet with 1mL pre-warmed complete medium and dispense into a 25 cm² culture flask containing 10mL pre-warmed complete medium. 4. Incubate the culture at 37°C incubator with 5% CO₂. <p>A subcultivation ratio of 1:4-1:8 is recommended. Cells should be passaged when cells grow splitting at 80-90% confluence.</p>
Cryopreservation Medium	Complete medium supplemented with 10% (v/v) DMSO.
Storage Condition	Stored in liquid nitrogen for a long time, less than -130°C.
Mycoplasma	Free

Validation Scheme:
T7 Endonuclease I mismatch
cleavage assay



Demonstration of stable Cas9 activity in the cell line.

Human 293T cells stably expressing Cas9 nuclease were transfected with EdiGene's plasmid carrying a sgRNA for a candidate gene. 24 hours post-transfection, genomic DNA was extracted from the collected cells. The PCR products flanking the sgRNA target site of 293T and 293T-Cas9 cell were treated with T7 Endonuclease I. The 293T without Cas9 shows a single band, while 293T-Cas9 has two extra bands which is a feature of active Cas9.

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EdiGene Inc. Life Science Park, 22 KeXueYuan Road, Changping District, Beijing 102206, P.R. China

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