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Cas9-expressing HeLa Cell Line-BSD

Catalog #	**EX0017567301**
Description	EdiGene's Cas9-expressing HeLa cell line-BSD has been developed by the integration of the CMV plasmid, which contains *OCT1* and 2A-linked *Cas9* genes as well as IRES-linked blasticidin resistant gene, into HeLa cell genome. This cell line is blasticidin resistant and has a high level constant expression of *Cas9* and *OTC1* that has been shown to boost U6 promoter-driven sgRNA expression. Combining with EdiGene's U6 promoter-containing sgRNA lentiviral plasmid, the cell line has been successfully used to generate hundreds of read-made 100% gene knockout cell lines validated by sequencing.
Plasmid Map	
Parental cell line	HeLa
Cell Type	Epithelial
Adherent/Suspension	Adherent
Organism	Homo sapiens
Amount	1×10^6 cells/tube, 1mL
Shipping Conditions	Dry ice
Complete Medium	DMEM, 10% FBS
Handing Procedure	Upon arrival, it should be stored in liquid nitrogen vapor phase and not at -80℃. Storage at -80℃ will result in loss of viability. 1. Thaw the vial in 37℃ 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 cm^2 culture flask containing 10mL pre-warmed complete medium. 4. Incubate the culture at 37℃ incubator with 5% CO_2. 5. A subcultivation ratio of 1:4-1:6 is recommended. Cells should be passaged when cells grow splitting at 80-90% confluence.
Cryopreservation Medium	Complete medium supplemented with 10% (v/v) DMSO.
Storage Condition	Stored in liquid nitrogen for a long time，less than -130℃.
Mycoplasma	Free

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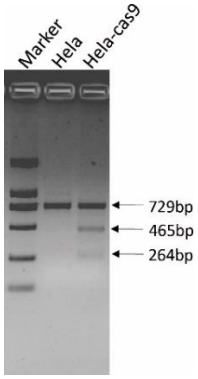
Validation Scheme:

T7 Endonuclease I mismatch cleavage assay



Demonstration of stable Cas9 activity in the cell line.

Human HeLa 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 HeLa and HeLa-Cas9 cell were treated with T7 Endonuclease I. The HeLa without Cas9 shows a single band, while Hela-Cas9 has two extra bands which is a feature of active Cas9.

References

Yuexin Z, Shiyou Z, Changzu C, et al. High-throughput screening of a CRISPR/Cas9 library for functional genomics in human cells[J]. Nature, 2014, 509(7501):487.

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