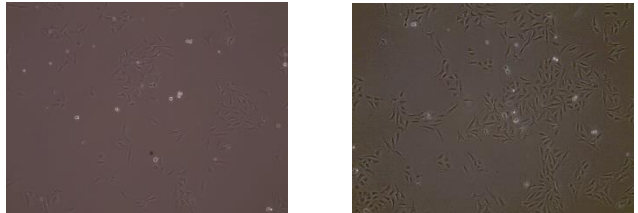


Product Information		
Cell Line	U2OS PLEC Knockout	
Parental	U2OS	
Product ID	U2OS-PLEC KO 10	
Product Batch	U2OS-PLEC-KO-210622	
Genotype	CRISPR/cas9-edited	
Passage	P6	
Date of Production	2020/09	
Properties		
Volume	1 ml/vial	
Storage Conditions	Liquid Nitrogen	
Cell Number/ Vial	0.7x10 <sup>6</sup> cells/ml	
Viability	85%	
Quality Control		
Test	Test Method	Pass/Fail
Viability	Post thawing culture	Pass
Mycoplasma	MycoAlert™ Mycoplasma Detection Kit (Lonza)	Pass
Cell Line Characterization	Sanger Sequencing (DNA)	Pass
Morphology Images	10x objective      24h Post-Thaw      48h Post-Thaw	
		
Growth Conditions		
Culture Media	Dulbecco's Modified Eagle's Medium (DMEM) supplemented with FBS 10%, L-glutamine 2mM, Penicillin-Streptomycin 100U/ml	
Passage Method	Trypsin	
Freezing Media	FBS with 10% DMSO	
Recommended Subculture	Cells are cultured as a monolayer at 37°C in a humidified atmosphere with 5% CO <sub>2</sub> . Cells should be passaged every 5-7 days. Split at 80-85% confluency, approximately 1:10-1:20.	
Cell Line Revival	Rapidly thaw cells in a 37°C water bath. Transfer contents into a tube containing 5 ml pre-warmed media. Centrifuge cells, remove supernatant wash cells with 10 ml PBS, centrifuge cells, remove PBS and seed into a 10 cm flask containing pre-warmed media.	

Cell Line Characterization (DNA)	
Target Gene	PLEC
Guide RNA Sequence	cttggtgaccacttggtga
Genomic Location	Chromosome 8: 143,915,147-143,976,734 reverse strand
Gene	ENSG00000178209
Target Protein	Plectin
Mutation	StopTag insertion in exon 3 of one chromosome (StopTag sequence: GCCGGCGTAACTAGCTGA). On another chromosome, there are 19bp insertion and 116bp deletion, which contains 34bp deletion of exon 3
<b>Forward Primer</b>	<b>Reverse Primer</b>
caggaccctcccatttctc	gaaaggtgagcacacagg
Sequence	
1). One chromosome sequence: 2). Another chromosome sequence: CTGGGTCTCTGCTGTGTGGCTGGGCTGCGGGTGAGGGCGCCTGGGTCTCAGTGTGTGATCTGAGGGAGGCTTTGGGGCTCCCA GGCAGGACCTGGCCAGCCAGTCAACAAGCACAAGTGGGTCAAGTGGGTCAACAAGCACCTCATCAAGTTGGTGGCGCATGCT GGTGCGTGTACAGGGGCTGAGGCTGTGGGAGGCGCTGAGGGAGGGTGGCCCTGCCGCCAAGGCTGTCCCATGCTGCCTG TGTGCTCACCTTTCATCTCTGAAACCTGTTATTTCTGTCTT	
Sequence Alignment	
1) WT 1 ccccaggtaaagctgggtcttctccccagatgagcgggatcgtgtgcaag 50     KO 1 CCCCAGGTAAGCTGGTCTTGTCTCCCCAGATGAGCGGGATCGTGTGCAG 50     51 aagaaaaaccttcaccaagtggtcaacaagcacctcatcaaggttggtgg 100     51 AAGAAAACCTGC-CGGCGTAACT-AGCTGACACCTCATCAAGGTTGGTGG 98	2) WT 1 ctgggtctctgctgtgtggctgggctgagggtgagggcgccctgggtctca 50     KO 1 CTGGGTCTCTGCTGTGTGGCTGGGCTGCGGGTGAGGGCGCCTGGGTCTCA 50     51 gtgtgtgatctgagggaggctttgggctccccagggcaggacctggccag 100     51 GTGTGTGATCTGAGGGAGGCTTTGGGCTCCCCAGGCAGGACCTGGCCAG 100     101 ccaggctgctgtgtctgcacgggtggtcacctgagggccccgggcttct 150     101 CCAG----- 104     151 cccagggcccaggtaaagctgggtcttctccccagatgagcgggatcgt 200     101 ----- 104     201 gtgcagaagaaaaacctca-----caagtggttcca 231     104 -----TCAACAAGCACAAGTGGGTCAAGTGGGTCCA 135     232 acaagcacctcatcaaggttggtggcgcacatgctggtgctgacagggg 281     135 ACAAGCACCTCATCAAGGTTGGTGGCGCATGCTGGTGCCTGTACAGGGG 184