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Datasheet for 451Lu-01-0005

451Lu Viable Cells

Overview

Description:	451Lu Viable Cells - 451Lu-01-0005
Item No.:	451Lu-01-0005
Size:	5 x 1 million cells
Applications:	Cellular Assay, Other, WB
Origin:	Human

Product Details

Background:	451Lu is a selected xenograft aggressive metastatic sub-line of WM164. WM164 is a metastatic human melanoma cell line established from a metastatic site (right upper arm) in a 22-year-old male with stage IV superficial spreading melanoma. 451Lu cell line features the specific V600E (Val600Glu) mutation at codon 600 in the BRAF gene. This mutation causes constitutively active kinase activity and activation of MEK and ERK signaling pathway. This cell line is wild type for PTEN, N-RAS, c-KIT, and CDK4 genes.
Synonyms:	Melanoma, patient derived tumor, tumor models, skin cancer, xenograft
Species of Origin:	Human

Target Details

Purity/Specificity:	Cells are sterile, validated by short tandem repeat profiling, and are tested as negative for mycoplasma. It is recommended that cell lines are tested for mycoplasma contamination and short tandem repeat (STR) profiling every 10 passages or each time a frozen seed stock is made. See cell culture protocol for additional details.
Relevant Links:	• Cell Line EULA • Melanoma Cell Culture Protocol

Application Details

Suggested Applications:	Cellular Assay, Other, WB (Based on references)

Application Note:	The key applications of these cell lines include genetic studies, xenograft production, drug testing, and drug target discovery. These cell line models can be used in various biological assays, and for identifying critical target genes, and cell signaling pathways.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.

Cell Line Data

Cell Line:	Human Melanoma
Product Type:	Viable Cells
Cell Viability:	Yes
Stage:	Metastasis
BRAF:	V600E
CDK4:	WT
C-Kit:	WT
N-RAS:	WT
PTEN:	WT
Paired:	Yes
Medium:	Tumor Specialized Media with 2% HI-FBS
Sub-culture:	Cells should be maintained between 30 – 95% confluence in tumor specialized medium with 2% FBS; split cultures 1:10 every 5-6 days using 0.25% trypsin/EDTA.
Incubation:	36°C with 5% CO2

Formulation

Physical State:	Frozen Cell Suspension
Concentration:	1.0 million cells/ml Count By Hemocytometer
Buffer:	None
Preservative:	None
Stabilizer:	None

Shipping & Handling

Shipping Condition:	Dry Ice

Storage Condition:	Cells are frozen with 90% FBS/10% DMSO solution at about 5x10^6 cells/ml. Store vial in liquid nitrogen upon arrival.
Expiration:	Expiration date is two (2) years from date of receipt.

Images

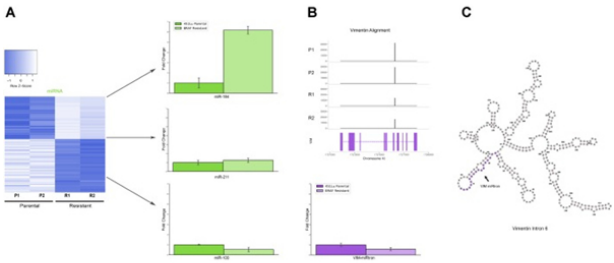
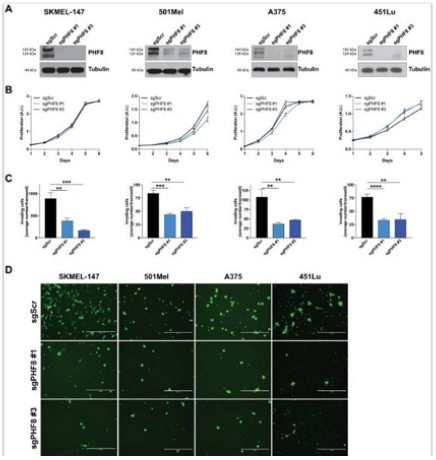
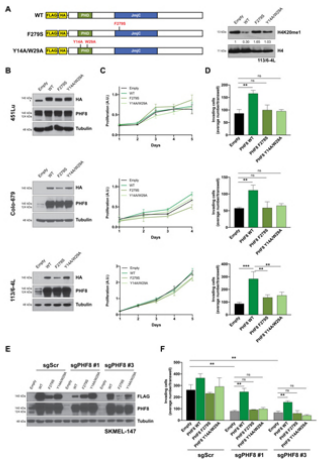


Figure
Detailed analysis miRNAs of interest. (A) qPCR representing fold change of miRNAs miR-184, miR-211, and miR-100 in 451Lu BRAF Resistant samples relative to 451Lu parental expression levels across replicates. (B) qPCR representing log fold change of the VIM miRNA in 451Lu BRAF Resistant vs. Parental samples and BAM gene alignment tracks across samples. (C) RNAfold predicted secondary structure of VIM intron 6 with the candidate miRNA highlighted in purple. Fig 4. PMID: 30349559

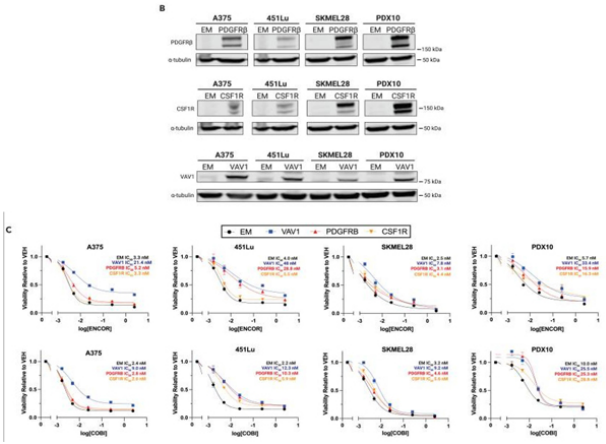


Western Blot
(A) Efficient PHF8 knockout using two different sgRNAs in four different melanoma cell lines was determined by Western blot. The membrane was reblotted with tubulin antibody for loading control. (B) Proliferation curves of cells seeded 3 days after infection with sgRNA-carrying lentiviral particles targeting PHF8. (C) Invasion assay of cells seeded 3 days after infection with sgRNA-containing lentiviral particles, using Fluoroblok transwell chambers coated with Matrigel. PHF8 knockout significantly inhibits melanoma invasion in SKMEL-147 (sgPHF8 #1 versus sgScr, P = 0.003; sgPHF8 #3 versus sgScr, P = 0.0007), 501Mel (sgPHF8 #1 versus sgScr, P = 0.0003; sgPHF8 #3 versus sgScr, P = 0.002), A375 (sgPHF8 #1 versus sgScr, P = 0.003; sgPHF8 #3 versus sgScr, P = 0.006), and 451Lu cells (sgPHF8 #1 versus sgScr, P = 0.0001; sgPHF8 #3 versus sgScr, P = 0.003). (D) Representative pictures of the Fluoroblok transwell inserts stained with Calcein AM to quantify invading cells in (C). Scale bars, 400 µm. Fig 2. PMID: 35179962

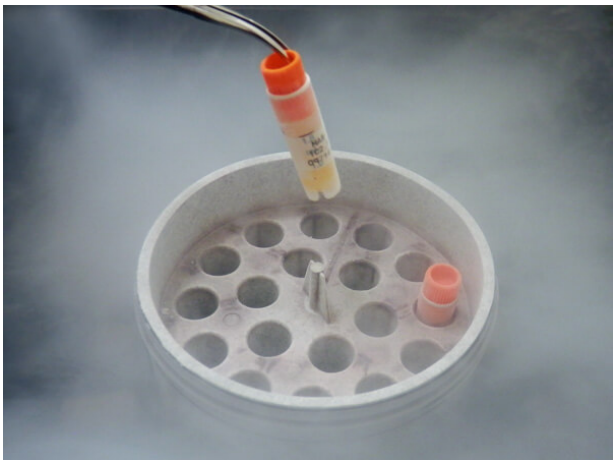


Western Blot

(A) Left: Lentiviral constructs encoding for FLAG-HA tagged PHF8 WT, F279S, and Y14A/W29A. Right: Immunoblotting of H4K20me1 in 113/6-4L overexpressing PHF8 WT or PHF8 mutants. H4 Western blot serves as loading control. Band intensity relative to Empty condition is shown. (B) Efficient overexpression of PHF8 WT and mutant constructs in three cell lines was determined by Western blot using HA and PHF8 antibodies. (C) Proliferation and (D) Invasion assays were performed in 451Lu (top: PHF8 WT versus Empty, P = 0.002; PHF8 F279S versus Empty, P = 0.42; PHF8 Y14A/W29A versus Empty, P = 0.43), Colo-679 (middle: PHF8 WT versus Empty, P = 0.004; PHF8 F279S versus Empty, P = 0.91; PHF8 Y14A/W29A versus Empty, P = 0.13), and 113/6-4L cells (bottom: PHF8 WT versus Empty, P = 0.0004; PHF8 F279S versus PHF8 WT, P = 0.002; PHF8 Y14A/W29A versus PHF8 WT, P = 0.005). (E) Efficient overexpression of PHF8 WT and mutant constructs in SKMEL-147 cells previously transduced with sgScr or sgPHF8 #3 was determined by Western blot. (F) Invasion assay shows that ectopic PHF8 WT expression, but not the mutant constructs, restores the invasive potential of PHF8 knockout SKMEL-147 cells (sgPHF8 #1 + Empty versus sgScr + Empty, P = 0.005; sgPHF8 #1 + PHF8 WT versus sgPHF8 #1 + Empty, P = 0.002; sgPHF8 #1 + PHF8 F279S versus sgPHF8 #1 + Empty, P = 0.14; sgPHF8 #1 + PHF8 Y14A/W29A versus sgPHF8 #1 + Empty, P = 0.089; sgPHF8 #3 + Empty versus sgScr + Empty, P = 0.004; sgPHF8 #3 + PHF8 WT versus sgPHF8 #3 + Empty, P = 0.004; sgPHF8 #3 + PHF8 F279S versus sgPHF8 #3 + Empty, P = 0.69; sgPHF8 #3 + PHF8 Y14A/W29A versus sgPHF8 #3 + Empty, P = 0.098). Error bars indicate average ± SD. Representative data of three independent experiments conducted are shown. Western blot membranes were reblotted with tubulin antibody for loading control. Fig 5. PMID: 35179962



Western Blot
(B) Representative western blot with indicated antibodies in a panel of melanoma cell lines engineered to overexpress the screen candidates, empty vector (EM), VAV1, PDGFRB, or CSF1R.
(C) Dose-response of modified cell lines against ENCOR and COBI. Top row shows ENCOR data and bottom row shows COBI data. Each data point represents three replicate measurements. Viability is shown after three days of drug treatment for A375 and 451Lu and four days for SKMEL28 and PDX10. The error bars denote the 95% confidence interval.
Fig 3. PMID: 37860756



Flask
Human melanoma tumor cells with known gene mutations, disease stage, STR, and RPPA profiling

References

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