

CELL PANEL

SMALL CELL LUNG CANCER P53 HOTSPOT MUTATION CELL PANEL

p53 is a tumor suppressor protein encoded by the TP53 gene that responds to DNA damage by regulating cell-cycle arrest, apoptosis and senescence. At least 50 % of human tumors contain mutations or deletions of the TP53 gene. The Small Cell Lung Cancer p53 Hotspot Mutation Cell Panel (ATCC® TCP-2040™) is composed of 6 select cell lines derived from the lung that have been sequenced and validated by ATCC. This panel includes p53 WT cell lines as well as cultures with p53 hotspot mutations at codons 175, 248, 249, or 273. The panel is useful for anti-cancer drug targeting or reactivation of mutant p53 as well as studies related to p53 molecular mechanisms.

ATCC® No.	Name	Tissue	Histology	Tumor Source	TP53 Status	Zygoty	CDS Mutation	AA Mutation
CRL-9609™	BEAS-2B	lung	normal tissue, SV-40 immortalized	NA	WT			
CRL-5903™	NCI-H1882	lung	small cell lung carcinoma	metastasis (bone marrow)	WT			
CRL-5869™	NCI-H1417	lung	small cell lung carcinoma	primary	MUT	homozygous	c.524G>T	p.R175L
CRL-5837™	NCI-H719	lung	small cell lung carcinoma	metastasis (bone marrow)	MUT	homozygous	c.743G>A	p.R248Q
CRL-5856™	NCI-H1105	lung	small cell lung carcinoma	metastasis (lymph node)	MUT	homozygous	c.747G>T	p.R249S
CRL-5853™	NCI-H1048	lung	small cell lung carcinoma	metastasis (pleural effusion)	MUT	heterozygous	c.817C>T1	p.R273C

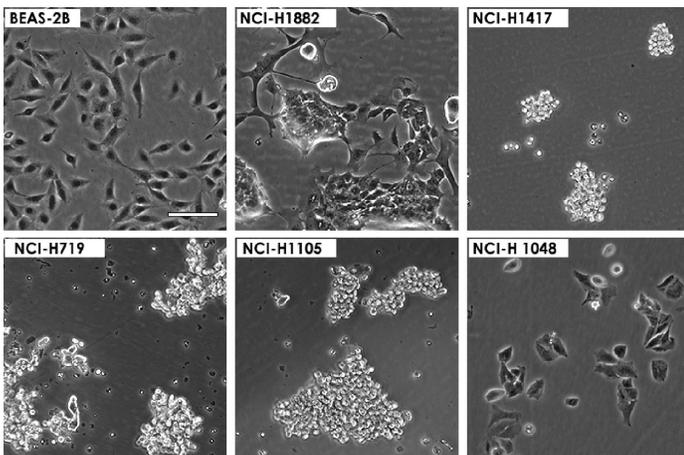


Figure 1: Cell morphology of six cell lines in the Small Cell Lung Cancer p53 Hotspot Mutation Cell Panel. Two p53 wild-type lung cell lines, BEAS-2B and NCI-H1882, and four p53 hotspot mutation lung cancer cell lines, NCI-H1417, NCI-H719, NCI-H1105, and NCI-H1048, were maintained in ATCC recommended culture conditions. Cell morphology was observed under Nikon™ microscopy, and images of the indicated cell lines were captured by Olympus® digital camera. Scale bar represents 100 μm.

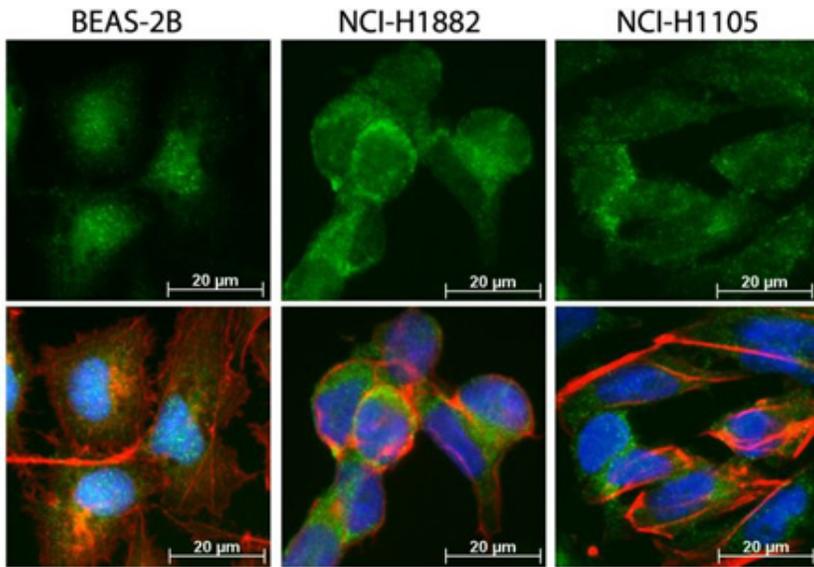


Figure 2: Immunofluorescence staining of p53. The indicated p53 wild-type and p53 mutation cells were grown on collagen-coated coverslips. Cells were fixed with 4% paraformaldehyde. p53 was stained with p53 primary antibody and Alexa Fluor 488 secondary antibody (green). F-actin was visualized with phalloidin Alexa Fluor 594 (red). Nuclei of the cells were visualized with Hoechst 33342 (blue). Single fluorescence channel images of p53 staining are shown in the upper row, and multichannel merged images are shown in the bottom row.

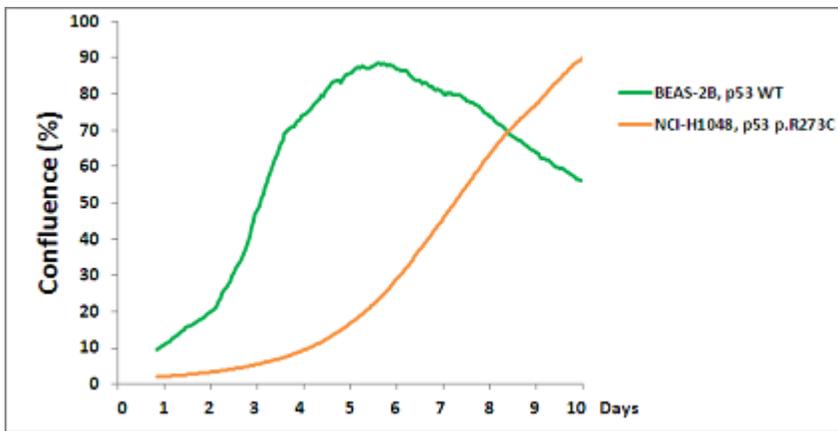


Figure 3: Cell growth kinetics. The indicated p53 wild-type and p53 mutation cells were cultured in ATCC recommended media, and plated at 3000 cells/well in 96-well plates. The cell growth kinetics were constantly monitored for 10 days using a label-free automated IncuCyte® live-cell imaging system (Essen Bioscience).

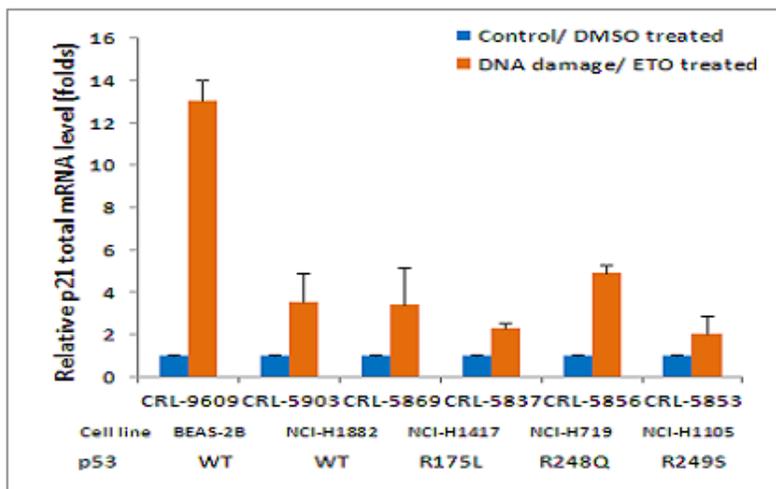


Figure 4: Real time PCR analysis of total mRNA levels of p21, a downstream target of p53, in the indicated p53 wild-type and p53 mutation cell lines. Cells were treated with 20 μm etoposide (ETO) for 6 hours to induce DNA damage, or treated with DMSO as a control. Total mRNA level of p21 and 36B4 were determined by real time quantitative PCR. Relative p21 total mRNA changes were normalized to the housekeeping gene 36B4.

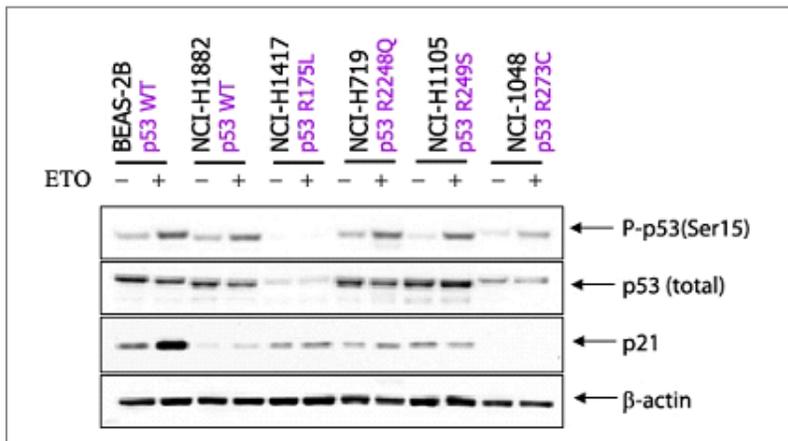


Figure 5: The indicated p53 wild-type and p53 mutation cells were treated with 20 μ m etoposide (ETO) for 8 hours to induce DNA damage, or treated with DMSO as a control. Western blotting assay was used to examine phosphorylation of p53 at Serine 15, total protein expression of p53, and expression of p21, a downstream target of p53. β -actin protein was also examined as a control.

Testing performed for each ATCC cell line was completed on current (2012) distribution material. ATCC provides these data in good faith, but makes no warranty, express or implied, nor assumes any legal liability or responsibility for any purpose for which the data are used. Nikon™ is a trademark of Nikon Corporation. Olympus® is a registered trademark of Olympus Corporation. IncuCyte™ is a trademark of Essen Instruments, Inc. The ATCC trademark and trade name, any and all ATCC catalog numbers, and any other trademarks listed are trademarks of the American Type Culture Collection unless indicated otherwise. ATCC products are intended for laboratory research only. They are not intended for use in humans, animals or diagnostics.