ATCC

CELL PANEL

COLON 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 Colon Cancer p53 Hotspot Mutation Cell Panel (ATCC® TCP-2020™) is composed of 6 select cell lines derived from colon cancer that have been sequenced and validated by ATCC. The panel includes both WT p53 cell lines as well as cultures with p53 hotspot mutations at codons 175, 245, 248, or 273. The panel is useful for novel 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	Zygosity	CDS mutation	AA mutation
CL-188™	LS174T	colon	adenocarcinoma	primary	WT	-	-	-
CCL-231™	SW48	colon	adenocarcinoma	primary	WT	-	-	-
CCL-255™	LS123	colon	adenocarcinoma	primary	MUT	homozygous	c.524G>A	p.R175H
CRL-2158™	LS1034	colon	adenocarcinoma	primary	MUT	homozygous	c.733G>A	p.G245S
CCL-220™	COLO 3200M	colon	adenocarcinoma	primary	MUT	homozygous	c.742C>T	p.R248W
CCL-218™	WiDr	colon	adenocarcinoma	primary	MUT	homozygous	c.818G>A	p.R273H

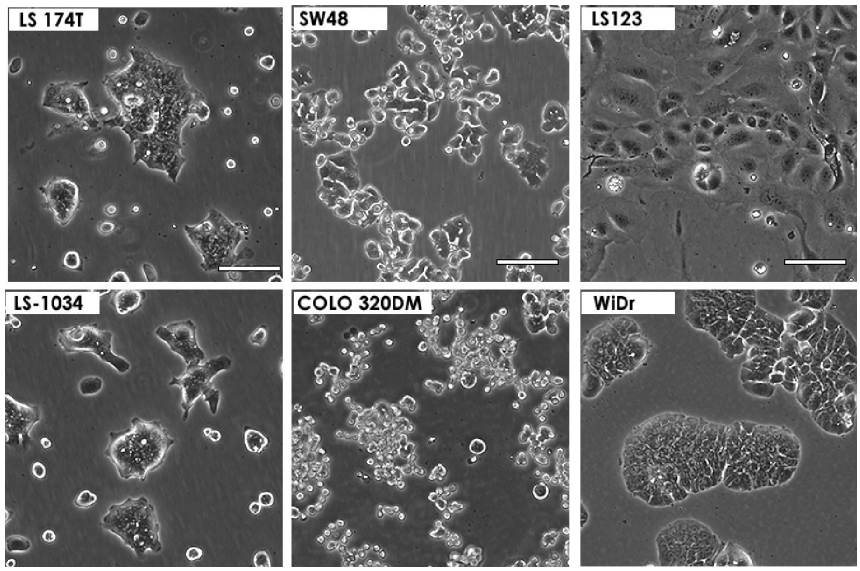

Figure 1: Cell morphology of six cell lines in the Colon Cancer p53 Hotspot Mutation Cell Panel. Two p53 wild-type colon cancer cell lines, LS174Tand SW48, and four p53 hotspot mutation colon cancer cell lines, LS123, LS1034, COLO 3200M, and WiDr, were maintained in ATCC recommended culture conditions. Cell morphology was observed under Nikon'" microscopy, and images of the indicated cell lines were captured by an Olympus® digital camera. Scale bar represents 100 µm.

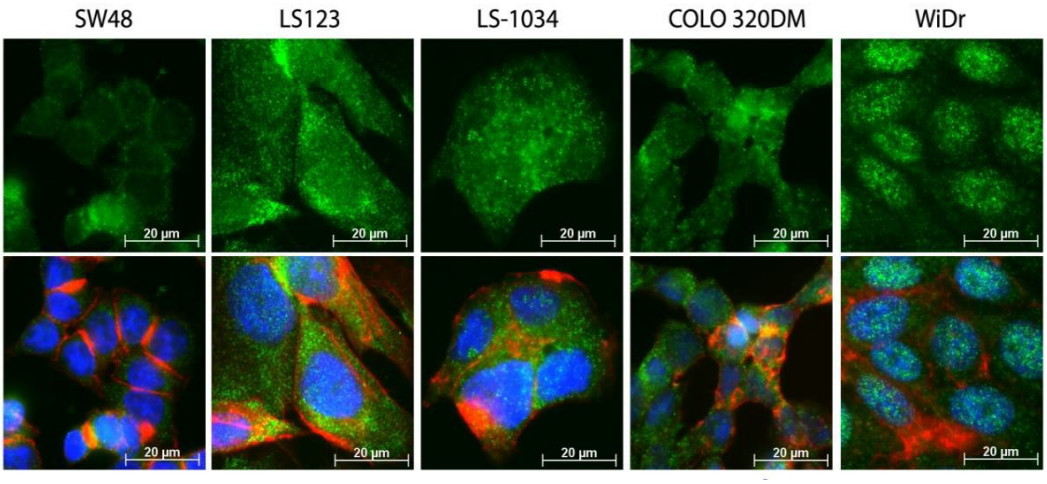

Figure 2: Immunofluorescence staining of p53. The indicated p53 wild-type and p53 mutation cells were grown on collagen-coated coverslips. Cells were fixed with 4% paraformaldehyde. p53 was stained with p53 primary antibody and Alexa Fluor 488 secondary antibody (green). F-actin was visualized with phalloidin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

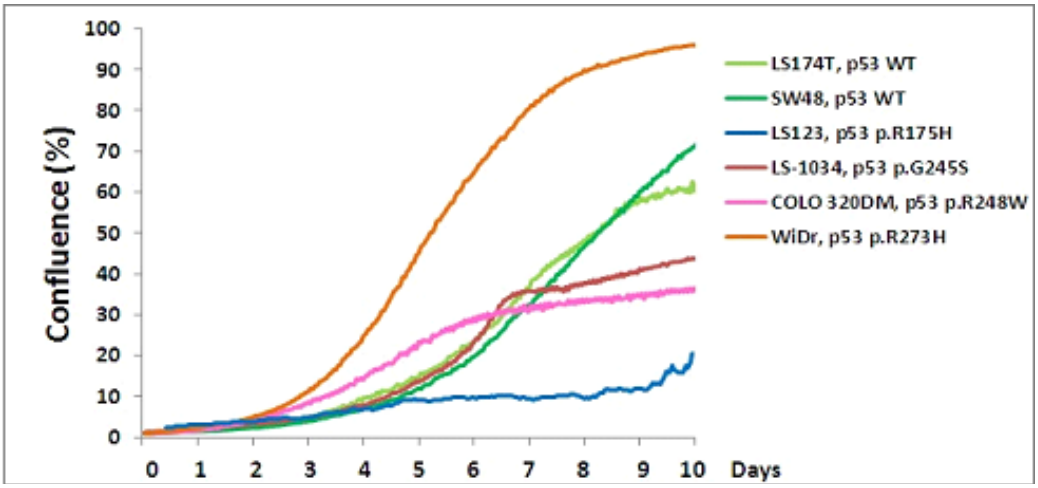

Figure 3: Cell growth kinetics. The indicated p53 wild-type and p53 mutation cells were cultured in ATCC recommended media, and plated at 3000 cells/well in 96-well plates. Cell growth kinetics were constantly monitored for 10 days using a label-free automated IncuCyte live-cell imaging system (Essen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

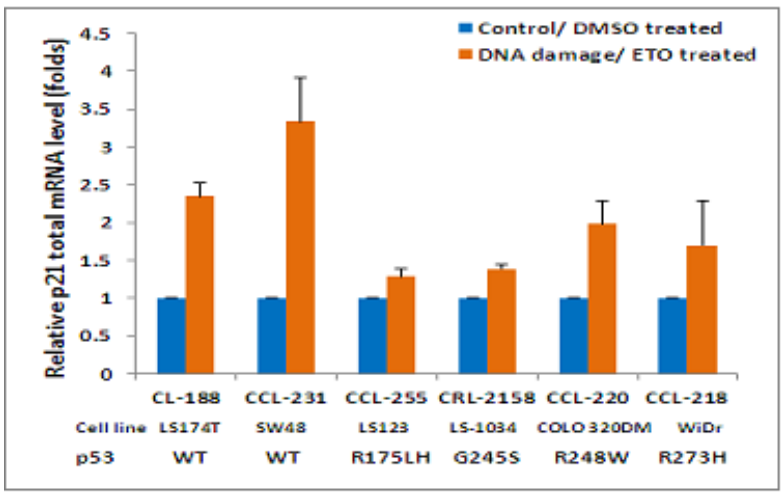

Figure 4: Real-time PCR analysis of total mRNA levels of p21, a downstream target of p53, in the indicated p53 wild-type and p53 mutation cell lines. Cells were treated with 20 µm etoposide (ETO) for 6 hours to induce DNA damage, or treated with DMSO as a control. Total mRNA levels of p21 and 36B4 were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

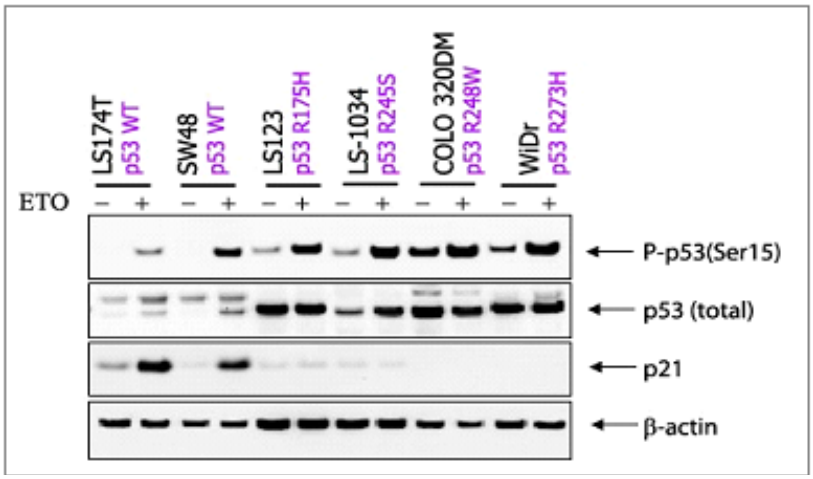

Figure 5: The indicated p53 wild-card and p53 mutation cells were treated with 20 μM etoposide (ETO) for 8 hours to induce DNA damage, or treated with DMSO as a control. Western blotting assay was used to examine phosphorylation of p53 at Serine 15, total protein express of p53, and expression of p21,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

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