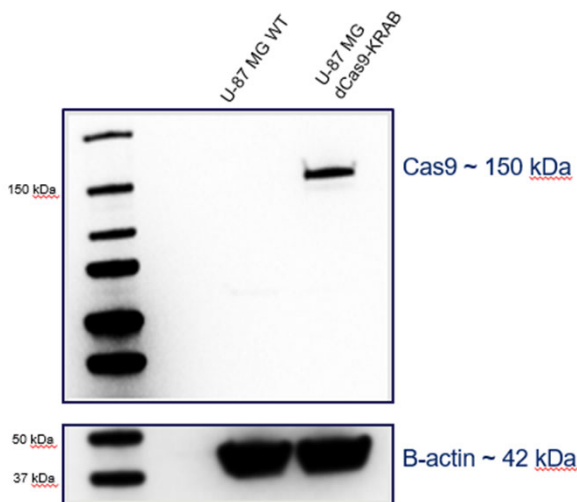


# Technical Data Sheet:

## U-87 MG dCas9-KRAB Cell Line

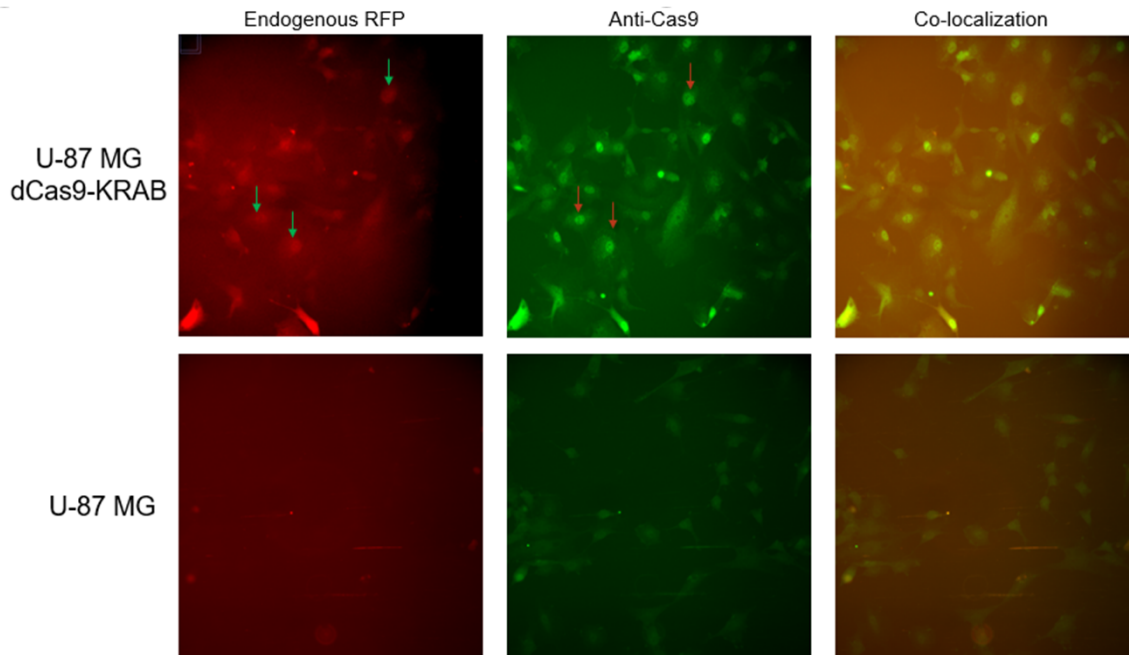
<b>ATCC® Number</b>	HTB-14dCas9-KRAB™
<b>Organism</b>	<i>Homo sapiens</i>
<b>Tissue/Disease Source</b>	Metastatic prostate adenocarcinoma
<b>Product Description</b>	<p>CRISPR interference (CRISPRi) has evolved as a powerful tool to interrogate gene function. While WT Cas9 protein has DNA binding and cutting activities, catalytically inactive Cas9 (dCas9), which carries point mutations in the two catalytic residues (D10A and H840A), retains only the DNA binding activity. When fused to a repression domain, such as the KRAB domain, dCas9 can function as a synthetic transcriptional repressor to interfere with gene expression.</p> <p>This cell line was created by knocking-in a KRAB-dCas9 (from <i>Streptococcus pyogenes</i>) expression cassette into the safe harbor AAVS1 locus using CRISPR/Cas9 gene editing technology. This cell line stably expresses KRAB-dCas9, RFP, and a hygromycin resistance gene. Functional evaluation of HTB-14dCas9-KRAB cells shows greater than 70% gene repression can be achieved for p53 and KRAS genes when their respective gRNAs were delivered into the cells.</p>
<b>Application</b>	This cell line can be used as a tool for loss-of-function genetic studies.

### Generation of U-87 MG dCas9-KRAB cell line



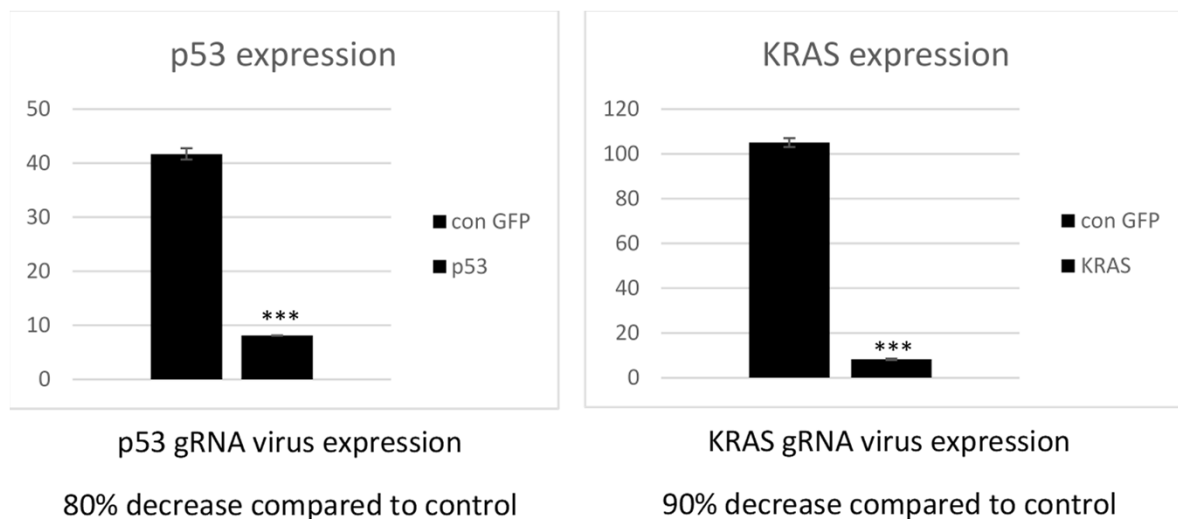
**Figure 1.** A) Schematic of AAVS1 dCas9-KRAB expression knock-in cassette, showing the RFP gene and hygromycin selection marker. B) Detection of dCas9 protein expression by Western blot in U-87 MG dCas9-KRAB cells, but not in U-87 MG parental cells.

## Generation of U-87 MG dCas9-KRAB cell line



**Figure 2.** Co-localization of dCas9 protein (green; middle) and RFP protein (red; left) in U-87 MG dCas9-KRAB cells. The overlaid image (right) indicates the co-expression of dCas9 and RFP in the same cells.

## Validation of gene expression knock down in U-87 MG dCas9-KRAB cells



**Figure 3.** Repression of p53 and KRAS gene expression. Lentivirus expressing gRNAs targeting p53 and KRAS gene were used, individually, to infect U-87 MG dCas9-KRAB cells. Lentivirus without gRNA expression was used as the control. 24 hours after infection, antibiotics were added to the culture media to enrich antibiotic resistant cells. Cell pellets were collected after 5 days of selection and subject to ddPCR gene expression quantification analysis. The expression of p53 and KRAS genes were significantly repressed in cells infected with gRNAs.

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