Product Specification Sheet

Product Name	**pAAVS1-SA-T2A-Neo-CAG-rtTA-pA-TRE-dCas9-KRAB-pA Donor Vector**
Description	CRISPR interference (CRISPRi) stands out as a powerful tool for targeted gene transcription silencing in both prokaryotic and eukaryotic cells. The collaboration between the inactive Cas9 (dCas9) and the Krüppel-associated box (KRAB) repressor ensures accurate and reversible gene silencing. Additionally, the strategic use of the Human AAVS1 "safe harbor" site, located within intron 1 of the PPP1R12C gene, minimizes its impact on cellular functions. Crucially, our approach incorporates the renowned Tet-On gene inducible system, providing precise control over gene expression. Responsive to specific signals, such as tetracycline, this system allows researchers to activate or deactivate genes as needed. Our innovative pAAVS1-SA-T2A-Neo-CAG-rtTA-pA-TRE-dCas9-KRAB-pA donor vector seamlessly integrates the strengths of CRISPRi, the AAVS1 safe harbor site, and the Tet-On gene inducible system. Featuring a TRE promoter-controlled dCas9-KRAB fusion gene, this design ensures manipulable and robust expression of the dCAS9/KRAB protein with tetracycline treatment, without disrupting cellular functions. The vector contains a CAG-rtTA cassette and will ensure tetracycline inducible expression of dCas9-KRAB from a tightly controlled TRE promoter. The vector strategically integrates a Neomycin selection marker with a splice acceptor (SA) site within the AAVS1 safe harbor locus, closely linking Neomycin-resistant gene expression to intron integration. This significantly reduces the risk of unintended off-target integrations during G418 selection. When combined with the SpCas9 nuclease and AAVS1 gRNA expression vector, the pAAVS1-SA-T2A-Neo-CAG-rtTApA-TRE-dCas9-KRAB-pA donor vector facilitates the seamless integration of a Tet-On-controlled dCas9/KRAB fusion protein into the AAVS1 safe harbor site. This approach, along with specific gene-targeted gRNAs, enables inducible, precise, and highly efficient repression of the target gene expression, providing an accessible and consistent method for specific gene silencing.
Catalog Number	AI7902
Size	10 µg at 0.5 µg/µL in TE
Shipping	Room temperature
Storage and Stability	Store at -20°C immediately upon receipt. This product is stable for 6 months when stored as directed.
Quality Control	This plasmid is sequence verified.

ALSTEM, INC
2600 Hilltop Drive, BLDG B, STE C328, Richmond, CA 94806
Tel: (510) 708-0096 Fax: (866) 605-8766
www.alstembio.com info@alstembio.com

Safety Precaution	This product does not contain any hazardous materials with occupational exposure limits. Nevertheless, ALSTEM strongly advises anyone handling this product to use suitable protective eyewear, such as chemical safety goggles or protective glasses, along with gloves and appropriate clothing to prevent skin contact.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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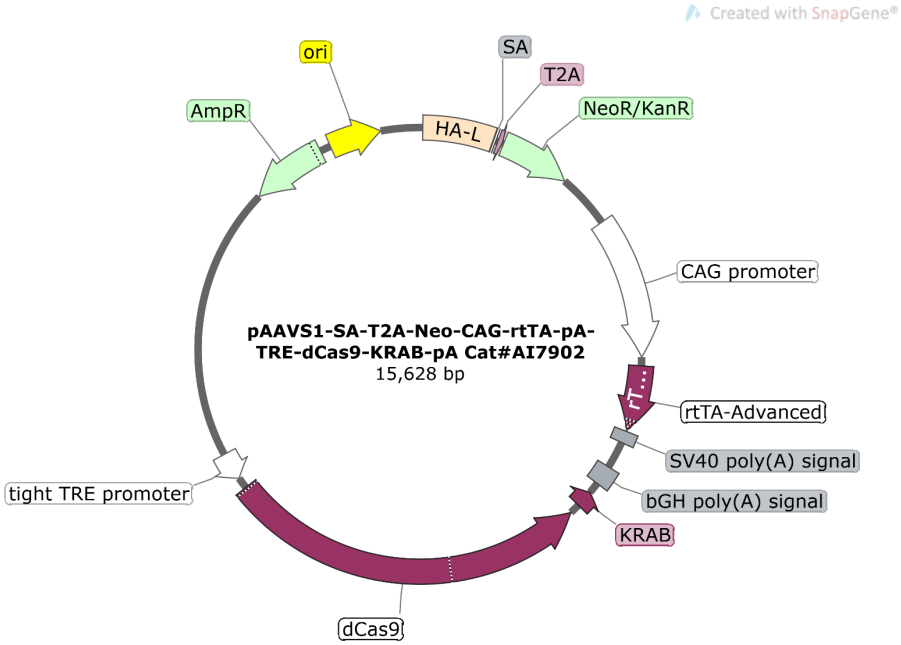
Vector Information

This AAVS1 donor vector includes essential components to serve as a reliable integration marker in the AAVS1 Safe Harbor site. It incorporates a universal SA-T2A-NeoR cassette, facilitating the integration of a dCas9/KRAB fusion gene driven by an inducible TRE promoter into the AAVS1 safe harbor site through antibiotic selection. This vector is suitable for generating inducible CRISPRi stable cell lines.



*Note: Bacterial culture of AAVS1 vectors should be done in medium containing **100 µg/mL** Carbenicillin. For maximal plasmid yield and quality, we recommend Stbl3 competent cells (Invitrogen).*

IMPORTANT NOTICE

Store the vial at -20°C immediately upon receipt.

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