

[Cat. No.] **ATS-0115**

Introduction

AccuTool™ Adeno-associated virus integration site 1 (AAVS1) donor kit is designed to integrate your gene of interest into human genome. AAVS1 is known as a safe harbor site in human chromosome 19. A safe harbor is a transcriptionally active region, so it can provide robust and stable transgene expression. Upon delivery of the AAVS1 donor vector, DNA double-strand breaks (DSBs) occur at the AAVS1 site, followed by DNA repair. Under the presence of AAVS1 Open reading frame (ORF) knock-in clones, targeted transgenes are integrated into AAVS1 site by homologous recombination.

Applications

- Genome editing
- Stable expression of genes and overexpression

Components

Components	Amount
dRGEN-HS_AAVS1-U6_SG	2 µg
pRGEN-Cas9-CMV/T7	5 µg
AAVS1 T7E1 forward primer	1 nmol
AAVS1 T7E1 reverse primer	1 nmol
AAVS1_donor vector	2 µg

* **Note:** For research use only. Not for use in diagnostic or therapeutic procedures.

Specifications

Expression vector for pre-built AAVS1 guide RNAs (dRGEN-HS_AAVS1-U6_SG), Cas9 gene (pRGEN-Cas9-CMV/T7), and HDR donor vector (AAVS1_donor vector) are ampicillin-resistance, and stable in general *E. coli* strains such as DH5α or XL1.

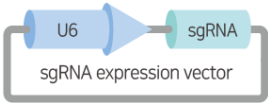


Figure 1. dRGEN-HS_AAVS1-U6_SG



Figure 2. pRGEN-Cas9-CMV/T7

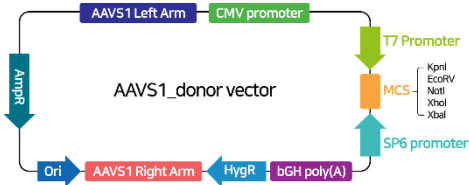


Figure 3. AAVS1_donor vector (6665bp)

Storage

- AccuTool™ AAVS1 donor kit components are lyophilized and delivered at ambient temperature.
- Store at -20°C after adding distilled water (D.W.) or TE buffer to the sample. Do not store it in a frost-free freezer.

Online Resources



Korean



English

Visit our [product page](#) for additional information and protocols.

Ordering Information

Description	Cat. No.
AccuTool™ AAVS1 donor kit	ATS-0115

Notice

Bioneer corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols

LOT Batch Code

REF Catalog Number

Caution

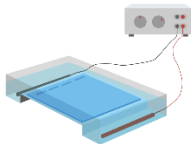
Consult Instructions For Use

RUO Research Use Only

Temperature Limitation

Use by Date

Experimental Procedures

Steps		Procedure Details
Gene knock-out cell establishment		
1	 Cloning into AAVS1_ donor vector	1. Insert your gene of interest using restriction enzyme sites (KpnI, EcoRV, NotI, XhoI, XbaI) in multiple cloning site (MCS) regions of the AAVS1_donor vector. * Note: The inserted gene sequence can be analyzed by Sanger sequencing with T7, CMV-F, or SP6 primers.
2	 Knock-in the transgenes into AAVS1 site	2. Prepare dRGEN-HS_AAVS1-U6_SG, pRGEN-Cas9-CMV/T7, and cloned AAVS1_donor vector with the final concentration of 3-5 µg/ µl in D.W. 3. We recommend using nucleofection kit for your specific cell line except for the DNA mixture. Recommended reaction mixture as follows. - dRGEN-HS_AAVS1-U6_SG: 2.5-5 µg - pRGEN-Cas9-CMV/T7: 2.5-5 µg - AAVS1_donor vector: 25-50 µg * Note: The amount of DNA given above is limited to the nucleofection kit and may be compatible with other methods, but the amount can vary. Since transfection efficiencies vary across different cell lines, we recommend optimizing for best results. We recommend starting with a 1:1 ratio. * Note: Transfection reagent can also use according to manufacturer's instructions.
3	 Isolation and analysis of AAVS1 knock-in clones	4. 48-96 hours after nucleofection, plate cells on culture dish with an appropriate amount of hygromycin. * Note: Recommended hygromycin concentration for human cells is 50-400 µg/ml. 5. Isolate monoclonal cell colonies after 2-4 weeks of plating. 6. Prepare genomic DNA from each clone between a 24 well plate and a 12 well plate (the plate wells may change depending on the purpose of the experiment). 7. To analyze donor integration into the AAVS1 site, perform junction-PCR analysis using primer set (Expected size: 846 bp, Tm=55°C). 8. Run the PCR reaction out on an approximately 1% agarose gel in 1X TBE buffer to confirm junction-PCR results.