AccuTool™ Rosa26 donor kit

[Cat. No.] ATS-0116

Introduction

AccuTool™ Rosa26 donor kit is designed to integrate your gene of interest in mouse genome. *Rosa26* is known as a safe harbor site in mouse chromosome 6. A safe harbor is a transcriptionally active region, so it can provide robust and stable transgene expression. Upon delivery of the *Rosa26* donor vector, DNA double-strand breaks (DSBs) occur at the *Rosa26* site, followed by DNA repair. Under the presence of *Rosa26* Open reading frame (ORF) knock-in clones, targeted transgenes are integrated into *Rosa26* site by homologous recombination.

Applications

- Genome editing
- Stable expression of genes and overexpression

Components

Components	Amount
dRGEN-Mm_Rosa26-U6_SG	2 μg
pRGEN-Cas9-CMV/T7	5 μg
Rosa26 T7E1 forward primer	1 nmol
Rosa26 T7E1 reverse primer	1 nmol
Rosa26_donor vector	2 μg

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

Specifications

Expression vector for pre-built *Rosa26* guide RNAs (dRGEN-Mm_Rosa26-U6_SG), Cas9 gene (pRGEN-Cas9-CMV/T7), and HDR donor vector (Rosa26_donor vector) are ampicillin-resistance, and stable in general *E. coli* strains such as DH5α or XL1.

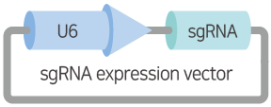


Figure 1. dRGEN-Mm_Rosa26-U6_SG

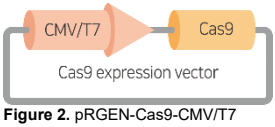


Figure 2. pRGEN-Cas9-CMV/T7

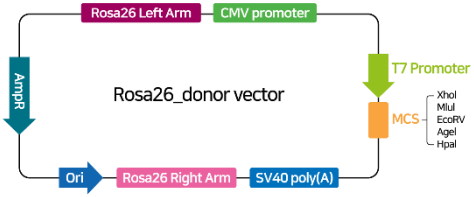


Figure 3. Rosa26_donor vector (4562bp)

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

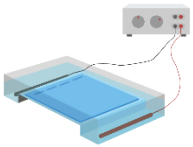
Description	Cat. No.
AccuTool™ Rosa26 donor kit	ATS-0116

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

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.