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.

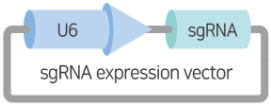


Figure 1. dRGEN-Mm_Rosa26-U6_SG

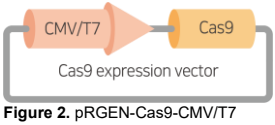


Figure 2. pRGEN-Cas9-CMV/T7

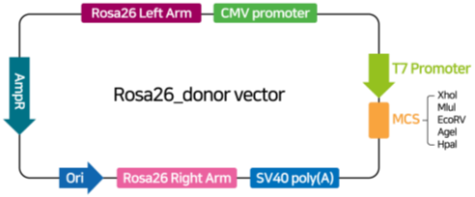


Figure 3. Rosa26_donor vector (4562bp)

Storage

- *AccuTool*™ Rosa26 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.

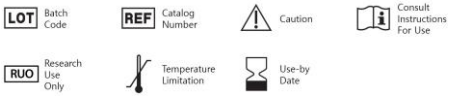
Ordering Information

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

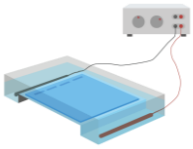
Notice

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 Rosa26_ donor vector**	1. Insert your gene of interest using restriction enzyme sites (XhoI, MluI, EcoRV, AgeI, HpaI) in multiple cloning site (MCS) regions of the Rosa26_donor vector. *** Note:** The inserted gene sequence can be analyzed by Sanger sequencing with T7 or CMV-F primers.
2	 **Knock-in the transgenes into *Rosa26* site**	2. Prepare dRGEN-Mm_Rosa26-U6_SG, pRGEN-Cas9-CMV/T7, and cloned Rosa26_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-Mm_Rosa26-U6_SG: 2.5-5 μg • pRGEN-Cas9-CMV/T7: 2.5-5 μg • Rosa26_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 be used according to manufacturer's instructions.
3	 **Isolation and analysis of *Rosa26* knock-in clones**	4. 48-96 hours after nucleofection, plate cells on culture dish. 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 *Rosa26* site, perform junction-PCR analysis using primer set (Expected size: 560 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.