ArciTect™ T7 Endonuclease I Kit

For estimation of CRISPR-Cas9 genome editing efficiency

Catalog # 76021 1 Kit 25 Reactions
76022 1 Kit 125 Reactions

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
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Product Description

ArciTect™ T7 Endonuclease I is the preferred enzyme for detecting genome editing such as insertions or deletions (INDELs) generated by CRISPR-Cas9. ArciTect™ T7 Endonuclease I Kit is comprised of ArciTect™ T7 Endonuclease I and ArciTect™ T7 Endonuclease I Buffer (10X), which have been tested and validated for use with the ArciTect™ CRISPR-Cas9 genome editing system. ArciTect™ T7 Endonuclease I recognizes and cleaves mismatched DNA, cruciform DNA structures, Holliday structures or junctions, heteroduplex DNA, and, less efficiently, nicked double-stranded DNA. Since the cleavage efficiency is proportional to the number of INDELs created at a specific DNA target, ArciTect™ T7 Endonuclease I Kit is used to estimate gene-editing efficiency in a rapid and cost effective manner.

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
ArciTect™ T7 Endonuclease I Kit	76021	1 Kit - 25 Reactions	• ArciTect™ T7 Endonuclease I (25 Reactions) • ArciTect™ T7 Endonuclease I Buffer (1 mL)*
ArciTect™ T7 Endonuclease I Kit	76022	1 Kit - 125 Reactions	• ArciTect™ T7 Endonuclease I (125 Reactions) • ArciTect™ T7 Endonuclease I Buffer (1 mL)*

*1 mL of buffer is sufficient for up to 500 reactions.

Component Storage and Stability

The following components are sold as part of ArciTect™ T7 Endonuclease I Kits (see Ordering Information) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
ArciTect™ T7 Endonuclease I	76023	25 Reactions	Store at -20°C.	Stable for 2.5 years from date of manufacture (MFG) on label.
ArciTect™ T7 Endonuclease I	76024	125 Reactions	Store at -20°C.	Stable for 2.5 years from date of manufacture (MFG) on label.
ArciTect™ T7 Endonuclease I Buffer (10X)	76025	1 mL	Store at -20°C.	Stable for 2.5 years from date of manufacture (MFG) on label.

Specifications

Source: E. coli

Concentration: 10 Units (U)/µL

Activity: Each Unit converts > 90% of 1 µg of supercoiled cruciform pUC (AT) plasmid DNA into linear DNA in 1 hour at 37°C.

Purity: > 95%

Cleavage Site: Single strand cleavage at the first, second, or third phosphodiester bond 5' to a DNA mismatch

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Genomic DNA isolation kit	e.g. Norgen Biotek 24700
PCR tubes	38091
Forward and reverse primers*	---
Nuclease-Free Water	79001
ArciTect™ High-Fidelity DNA Polymerase Kit	76026
Thermocycler	---
PCR purification kit	e.g. QIAGEN 28104
Microvolume spectrophotometer	---
Proteinase K Solution	79016
DNA Loading Dye	79018
1 kb DNA Ladder	79017
Agarose gel apparatus and reagents	---

*Forward and reverse primers should be designed to amplify a ~1000 base pair region surrounding the target site, offset so that T7 digestion will result in two fragments of distinctly different sizes for resolution on an agarose gel (e.g. 700 and 300 base pair fragments). For further information, refer to the Technical Bulletin: Evaluation of Genome Editing (Document #27126), available at www.stemcell.com or contact us to request a copy.

Directions for Use

1. Isolate genomic DNA (gDNA) from edited cells using a genomic DNA isolation kit.
2. Prepare Reagent Mix for PCR amplification of target region from 100 ng of gDNA as indicated in Table 1.

   NOTE: Indicated reaction volumes are for ArciTect™ High-Fidelity DNA Polymerase Kit. For other DNA polymerases, adjust component concentrations as required.

Table 1. Reagent Mix for PCR Amplification of Target Region

COMPONENT	VOLUME (µL)	FINAL AMOUNT/CONCENTRATION
ArciTect™ High-Fidelity Buffer OR ArciTect™ High GC Content Buffer	10	1X $MgCl_2$: 1.5 mM*
dNTP Mix (10 mM)	1	200 µM each
10 µM Forward primer	1 - 2.5 µL**	0.2 - 0.5 µM
10 µM Reverse primer	1 - 2.5 µL**	0.2 - 0.5 µM
DNA template	Variable	50 - 250 ng†
ArciTect™ High-Fidelity DNA Polymerase	0.5	1 U††
Nuclease-free water	Variable	Bring solution to total volume of 50 µL

*If desired, increase [$MgCl_2$] in 0.2 µM increments, up to 3.0 mM; [$MgCl_2$] > 3.0 mM may reduce fidelity.
**Use up to 5 µL (1 µM final concentration); 1 µL (0.2 µM) is recommended for most applications, while 2.5 µL (0.5 µM) is recommended for amplification of the HPRT positive control region.
†For low-complexity genomes (e.g. plasmid, virus, or bacterial artificial chromosome), 1 pg - 10 ng is recommended.
††For long targets (> 1 kb), difficult templates, or for higher yield, use up to 2 U polymerase.

3. Amplify the target region by PCR, using the conditions indicated in Table 2.

Table 2. PCR Cycling Conditions for Amplification of Target Region

STEP	TEMPERATURE	TIME
Initial denaturation	98°C	30 seconds *to 3 minutes**
Denaturation, annealing, extension for 15 - 35 cycles	98°C	5 - 10 seconds
	Varies** (annealing)	10 - 30 seconds
	72°C	15 - 30 seconds per kilobyte of DNA
Final extension	72°C	5 - 10 minutes
Hold	4°C	Up to 24 hours

*For difficult templates, initial denaturation can be extended up to 3 minutes.
**For primers over 20 nucleotides long, the annealing temperature should be ~3°C higher than the lowest melting temperature (Tm) of the primers. For primers shorter than 20 nucleotides, the annealing temperature should equal the Tm of the lowest primer. If the Tm of the primer pairs is ≥ 72°C, the annealing temperature and extension steps can be combined into a two-step cycling program. For amplification of the HPRT positive control region using ArciTect™ Human HPRT Primer Mix (included with Catalog #76013), an annealing temperature of 67°C is recommended.

4. Extract PCR product using a PCR purification kit, then measure the concentration using a microvolume spectrophotometer.
5. Transfer 200 ng of PCR product to a new PCR tube, and adjust to a final volume of 17 µL with nuclease-free water. Add 2 µL of ArciTect™ T7 Endonuclease I Buffer (10X).

   NOTE: If an uncut control is desired, prepare an identical second tube. This control will demonstrate that PCR generates a band of the expected size, which is only cleaved in the presence of T7 Endonuclease I.
6. Denature and anneal the PCR product in a thermocycler using the conditions indicated in Table 3.

   NOTE: If a thermocycler with the indicated ramp rates is unavailable, heat to 95°C for 10 minutes and let samples cool to room temperature (15 - 25°C).

Table 3. Denaturing and Annealing Conditions for PCR Products

STEP	TEMPERATURE	RAMP RATE	TIME
Initial denaturation	95°C	N/A	5 minutes
Annealing	95 - 85°C	-2°C/second	---
	85 - 25°C	-0.1°C/second	---
Hold	4°C	N/A	Infinite

N/A: Not applicable.

7. Add 1 µL of ArciTect™ T7 Endonuclease I. Incubate at 37°C for 15 minutes.

   NOTE: Do not add ArciTect™ T7 Endonuclease I to the uncut control tube.

   NOTE: Avoid an incubation temperature above 42°C, as this may cause an increase in non-specific nuclease activity.
8. Terminate the ArciTect™ T7 Endonuclease I reaction by adding 1 µL of Proteinase K Solution and incubate at 37°C for 5 minutes.

   NOTE: If not used immediately, store at -20°C. Thaw at room temperature (15 - 25°C) prior to use.
9. Perform gel electrophoresis by loading each reaction product with DNA Loading Dye into individual wells of a 1% agarose gel.
10. Using a gel imager, quantify cut (edited) and uncut (non-edited) band intensities for the digested condition(s). Using these values, estimate cleavage efficiency for each edited sample as follows:

    Cleavage efficiency (%) = (cut band intensity ÷ sum of cut and uncut band intensities) x 100

    NOTE: Band intensity, due to dye intercalation, is proportional not only to the number of DNA copies, but also to DNA length. As a result, the smaller cleavage product may be difficult to quantify and can be excluded from this estimate. Moreover, since ArciTect™ T7 Endonuclease I does not recognize single base pair mismatches or homozygous mutations, the actual efficiency of genome editing may be higher than estimated.

    See Figure 1 for representative examples of INDEL detection by T7 endonuclease I assay on agarose gels.

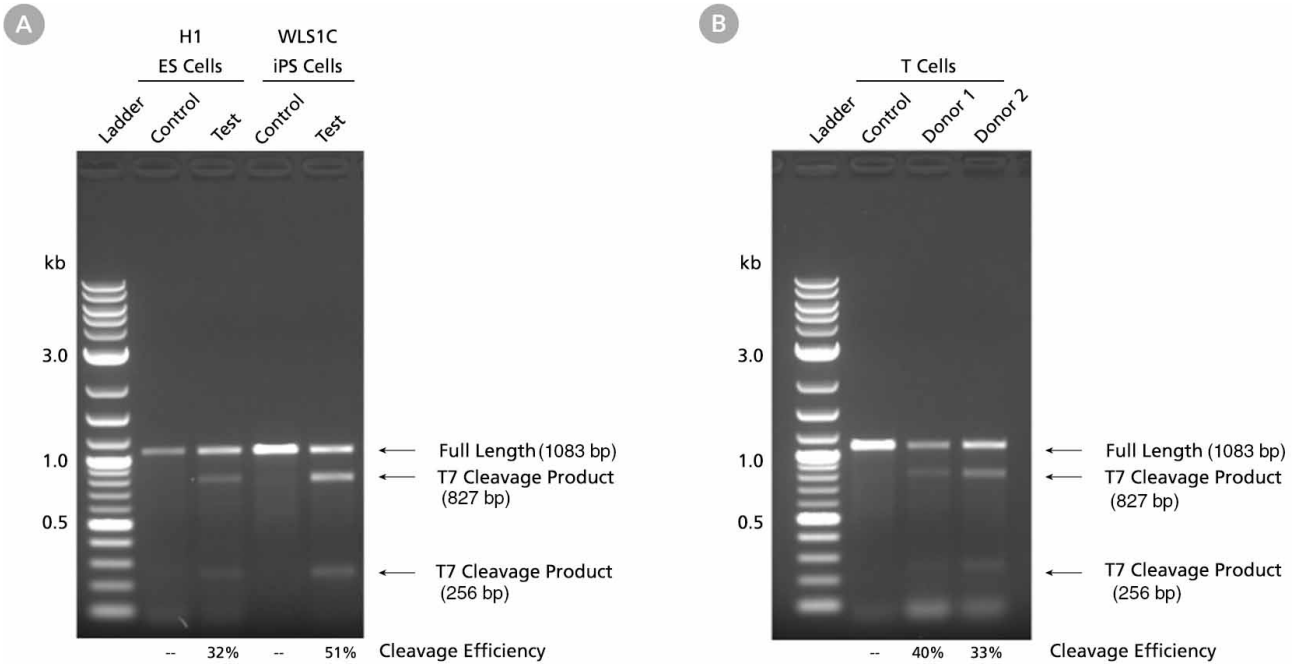

Figure 1. INDEL Detection by T7 Endonuclease I Assay. Human embryonic stem (ES) and induced pluripotent stem (iPS) cells (A) and T cells (B) were edited using ArciTect™ Cas9 Nuclease (Catalog #76002) and ArciTect™ Human HPRT Positive Control Kit (Catalog #76013), and INDEL formation was assessed using ArciTect™ T7 Endonuclease I Kit. Following CRISPR-mediated editing at the HPRT locus, genomic DNA was isolated and a 1 kb region surrounding the target site was amplified by PCR using ArciTect™ Human HPRT Primer Mix (included with Catalog #76013). PCR products were purified, then denatured, re-annealed, and cut with ArciTect™ T7 Endonuclease I. Samples were resolved on a 1% agarose gel, and band intensities were determined using a ChemiDoc™ MP Imaging System (Bio-Rad). Relative intensities of the full length (1083 base pairs [bp]) and T7 cleavage product bands (827 and 256 bp) were used to calculate the cleavage efficiency (%).
Control: Uncut PCR product (no T7 added); Test, Donor 1, and Donor 2: T7-digested PCR product.

Related Products

For related products, including other genome editing tools, specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com or contact us at techsupport@stemcell.com.

References

Mean RJ et al. (2004) Modification of the enzyme mismatch cleavage method using T7 endonuclease I and silver staining. Biotechniques 36(5): 758–60.

Vouillot L et al. (2015) Comparison of T7E1 and surveyor mismatch cleavage assays to detect mutations triggered by engineered nucleases. G3 (Bethesda) 5(3): 407–15.