

ArciTect™ Human HPRT Positive Control Kit

Positive control for CRISPR-Cas9 genome editing

Catalog # 76013 1 Kit



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Product Description

ArciTect™ Human HPRT Positive Control Kit is designed as a positive control for experiments using the ArciTect™ CRISPR-Cas9 genome editing system. The kit comprises ArciTect™ Human HPRT crRNA (2 nmol) and ArciTect™ Human HPRT Primer Mix (2 nmol), both of which have been tested and validated for use with the ArciTect™ line of genome editing products. HPRT, or hypoxanthine phosphoribosyltransferase, is a housekeeping gene and a commonly used control. The kit can be used to optimize transfection protocols and act as a positive control that can be used alongside custom ArciTect™ crRNAs (e.g. Catalog #76010). ArciTect™ Human HPRT crRNA first requires annealing to ArciTect™ tracrRNA (Catalog #76016) then must be combined with an ArciTect™ Cas9 Nuclease (e.g. Catalog #76002) to form a ribonucleoprotein complex. ArciTect™ Human HPRT Primer Mix can be used to amplify genomic DNA isolated from a population of transfected cells, which can subsequently be used in a T7 endonuclease I assay to determine cleavage genome editing efficiency.

Product Information

The following components are sold as a kit and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
ArciTect™ Human HPRT crRNA	76014	2 nmol	Store at -80°C. Alternatively, store at -20°C for up to 6 months.	Stable for 24 months from date of manufacture (MFG) on label.
ArciTect™ Human HPRT Primer Mix	76015	2 nmol	Store at -20°C.	Stable for 24 months from date of manufacture (MFG) on label.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Genomic DNA isolation kit	e.g. Norgen Biotek 24700
PCR tubes	38091
Nuclease-Free Water	79001
ArciTect™ High-Fidelity DNA Polymerase Kit <ul style="list-style-type: none">ArciTect™ High-Fidelity DNA PolymeraseArciTect™ High-Fidelity BufferArciTect™ High GC Content BufferdNTP Mix (10 mM)	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	---

Directions for Use

A. PREPARATION OF ArciTect™ HUMAN HPRT PRIMER MIX

- Briefly centrifuge the vial of ArciTect™ Human HPRT Primer Mix before opening.
- Add 20 μL of nuclease-free water. Mix thoroughly. This is a 100 μM stock solution.
NOTE: If not used immediately, aliquot and store at -20°C to -80°C for up to 6 months. After thawing the aliquots, use immediately. Do not re-freeze.
- Prepare a 10 μM working solution by diluting the 100 μM stock solution 1 in 10. Mix thoroughly.

B. PCR AMPLIFICATION OF gDNA FROM EDITED CELLS

- Edit cells using ArciTect™ Human HPRT crRNA. For further information, refer to the Technical Bulletin: Genome Editing of Human Pluripotent Stem Cells (Document #27084), available at www.stemcell.com or contact us to request a copy.
- Isolate genomic DNA (gDNA) from edited cells using a genomic DNA isolation kit.
- 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 GC Content Buffer	10	1X
dNTP Mix (10 mM)	1	200 μM each
10 μM ArciTect™ Human HPRT Primer Mix (working solution)	2.5 μL	0.5 μM
DNA template	Variable	100 ng
ArciTect™ High-Fidelity DNA Polymerase	0.5	1 U
Nuclease-free water	Variable	Bring solution to total volume of 50 μL

- 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
Denaturation, annealing, extension for 35 cycles	98°C	10 seconds
	67°C (annealing)	15 seconds
	72°C	45 seconds
Final extension	72°C	5 minutes
Hold	4°C	Up to 24 hours

- Extract PCR product using a PCR purification kit, then measure the concentration using a microvolume spectrophotometer.
- Proceed with the T7 endonuclease I assay using ArciTect™ T7 Endonuclease I Kit (Catalog #76021), as described in the corresponding Product Information Sheet (Document #DX21663), available at www.stemcell.com or contact us to request a copy.

For complete instructions on CRISPR-Cas9 genome editing, including annealing tracrRNA and crRNA to generate guide RNA, formation of the ribonucleoprotein (RNP) complex, and transfection into target cells, refer to the Technical Bulletin: Genome Editing of Human Pluripotent Stem Cells (Document #27084), available at www.stemcell.com or contact us to request a copy.

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

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- Hultquist JF et al. (2016) A Cas9 ribonucleoprotein platform for functional genetic studies of HIV-host interactions in primary human T cells. *Cell Rep* 17(5): 1438–52.
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- Liang X et al. (2015) Rapid and highly efficient mammalian cell engineering via Cas9 protein transfection. *J Biotechnol* 208: 44–53.
- Ran FA et al. (2013) Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell* 154(6): 1380–9.
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