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 • ArciTect™ High-Fidelity DNA Polymerase • ArciTect™ High-Fidelity Buffer • ArciTect™ High GC Content Buffer • dNTP 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

1. Briefly centrifuge the vial of ArciTect™ Human HPRT Primer Mix before opening.
2. 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.
3. 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

1. 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.
2. Isolate genomic DNA (gDNA) from edited cells using a genomic DNA isolation kit.
3. 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

4. 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

5. Extract PCR product using a PCR purification kit, then measure the concentration using a microvolume spectrophotometer.
6. 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.

Kim S et al. (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. Genome Res 24(6): 1012–9.

Liang X et al. (2015) Rapid and highly efficient mammalian cell engineering via Cas9 protein transfection. J Biotechnol 208: 44–53.

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