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), ArciTect™ Human HPRT Primer Mix (2 nmol), ArciTect™ tracrRNA (5 nmol), and ArciTect™ Annealing Buffer (5X). ArciTect™ Human HPRT crRNA first requires annealing to ArciTect™ tracrRNA (included in the kit). This must then 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 efficiency.

This kit has 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).

Product Information

The following components are sold as a complete 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 2 years from date of manufacture (MFG) on label.
ArciTect™ Human HPRT Primer Mix	76015	2 nmol	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
ArciTect™ tracrRNA	76016A	5 nmol	Store at -80°C. Alternatively, store at -20°C for up to 6 months.	Stable for 2 years from date of manufacture (MFG) on label.
ArciTect™ Annealing Buffer (5X)	76019	100 µL	Store at -20°C. Alternatively, store at 2 - 8°C for up to 6 months.	Stable for 2 years from date of manufacture (MFG) on label.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
ArciTect™ Cas9 Nuclease (or variant)	76002 (or 76009)
Genomic DNA Purification Kit	79020
PCR tubes	e.g. 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
Gel and PCR Cleanup Kit	79030
Proteinase K Solution	79016
DNA Loading Dye	79018
1 kb DNA Ladder	79017

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 to prepare a 100 µM stock solution. Mix thoroughly.

   NOTE: If not used immediately, aliquot and store at -20°C for up to 6 months.
3. Prepare a 10 µM working solution by diluting the 100 µM stock solution 1 in 10. Mix thoroughly.

B. PREPARATION OF ArciTect™ HUMAN HPRT crRNA AND ArciTect™ tracrRNA STOCK SOLUTIONS

1. Prepare stock solutions of ArciTect™ Human HPRT crRNA and ArciTect™ tracrRNA by centrifuging the vials before opening and adding nuclease-free water to give a final concentration of 200 µM, as indicated in Table 1.

Table 1. Preparation of crRNA and tracrRNA Stock Solutions

COMPONENT	AMOUNT OF RNA	VOLUME OF NUCLEASE-FREE WATER
ArciTect™ Human HPRT crRNA	2 mol	10 µL
ArciTect™ tracrRNA	5 nmol	25 µL

2. Mix thoroughly. If not used immediately, aliquot and store at -80°C for up to 6 months. After thawing the aliquots, use immediately. Do not re-freeze.

C. GENOME EDITING OF CELLS WITH HPRT POSITIVE CONTROL

For further information, refer to the Technical Bulletin: Genome Editing of Human Pluripotent Stem Cells (Document #27084) or Genome Editing of Human Primary T Cells (Document #27155), available at www.stemcell.com or contact us to request a copy.

1. Prepare 80 µM gRNA by combining components in a microcentrifuge tube as indicated in Table 2. Mix thoroughly.

Table 2. Preparation of 80 µM* gRNA

COMPONENT	VOLUME
ArciTect™ Human HPRT crRNA	4 µL
ArciTect™ tracrRNA	4 µL
ArciTect™ Annealing Buffer (5X)	2 µL
Total	**10 µL**

*80 µM is equivalent to 80 pmol/µL

2. In a thermocycler or heating block, incubate gRNA mixture at 95°C for 5 minutes followed by 60°C for 1 minute. Cool to room temperature (15 - 25°C) and place on ice.

   NOTE: If not used immediately, store at -80°C for up to 6 months.
3. Prepare RNP Complex Mix by combining ArciTect™ Cas9 Nuclease (4 µg/µL or 25 µM) with 80 µM gRNA (prepared in steps 1 - 2) in an appropriate transfection buffer.

   NOTE: For electroporation reactions, we recommend 1 - 4 µM Cas9 (final concentration in electrolytic buffer), and for chemical transfection reactions we recommend 10 - 100 nM Cas9 (final concentration in plating media). For both electroporation and chemical transfection methods, a 1:2 - 1:8 molar ratio of Cas9:gRNA is recommended. RNP complex formation must be optimized for cell type and transfection method.
4. Incubate RNP Complex Mix at room temperature (15 - 25°C) for 10 - 20 minutes.
5. Transfect cells using your preferred method.
6. Culture cells for 48 - 72 hours after transfection to allow genome editing to occur.

D. PCR AMPLIFICATION OF GENOMIC DNA FROM EDITED CELLS

1. Isolate genomic DNA (gDNA) from edited cells using the Genomic DNA Purification Kit.
2. Prepare Reagent Mix for PCR amplification of target region from 100 ng of gDNA as indicated in Table 3.

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

Table 3. 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

3. Amplify the target region by PCR, using the conditions indicated in Table 4.

Table 4. 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

4. Extract PCR product using the Gel and PCR Clean-up Kit, then measure the concentration using a microvolume spectrophotometer.

   OPTIONAL: Run product on a 1% agarose gel for visualization. Expected band size is 1083 base pairs (bp).
5. Proceed with the T7 endonuclease I assay using ArciTect™ T7 Endonuclease I Kit (Catalog #76021), as described in the corresponding Product Information Sheet, available at www.stemcell.com or contact us to request a copy.

   NOTE: After cleavage with T7 endonuclease I, expected cut band sizes are 827 and 256 bp.

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.