ArciTect™ Human CRISPR Optimization Kit

Complete kit for optimization of genome editing in human cells

Catalog # 100-0470 APC, 25 tests
100-0471 PE, 25 tests
100-0472 FITC, 25 tests

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Product Description

ArciTect™ Human CRISPR Optimization Kit is designed to enable optimization of genome editing in human cells. It can also be used as a positive control for experiments using the ArciTect™ CRISPR-Cas9 genome editing system in human cells. The kit comprises ArciTect™ Human B2M sgRNA, ArciTect™ Cas9 Nuclease, and a fluorophore-conjugated beta-2 microglobulin (B2M) antibody. ArciTect™ Cas9 Nuclease must first be complexed with ArciTect™ Human B2M sgRNA, followed by delivery into human cells and subsequent culture for 48 - 96 hours. Following culture, editing efficiency can be assessed by collection and processing for flow cytometry using a fluorophore-conjugated B2M antibody.

This kit has been tested and validated for use with the ArciTect™ line of genome editing products. By targeting the B2M gene that encodes a ubiquitously expressed cell-surface protein, editing efficiency can be rapidly and quantitatively assessed using flow cytometry methods. This technique also enables additional evaluation of editing success such as viability monitoring and/or assessment of cell type-specific marker expression.

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
ArciTect™ Human CRISPR Optimization Kit, APC	100-0470	25 tests	• ArciTect™ Human B2M sgRNA, 2 nmol (Component #200-0126) • ArciTect™ Cas9 Nuclease, 100 µg (Catalog #76002) • Anti-Human Beta-2 Microglobulin Antibody, Clone 35, APC, 25 tests (Component #100-0407)
ArciTect™ Human CRISPR Optimization Kit, PE	100-0471	25 tests	• ArciTect™ Human B2M sgRNA, 2 nmol (Component #200-0126) • ArciTect™ Cas9 Nuclease, 100 µg (Catalog #76002) • Anti-Human Beta-2 Microglobulin Antibody, Clone 35, PE, 25 tests (Component #100-0408)
ArciTect™ Human CRISPR Optimization Kit, FITC	100-0472	25 tests	• ArciTect™ Human B2M sgRNA, 2 nmol (Component #200-0126) • ArciTect™ Cas9 Nuclease, 100 µg (Catalog #76002) • Anti-Human Beta-2 Microglobulin Antibody, Clone 35, FITC, 25 tests (Component #100-0409)

Component Storage and Stability

COMPONENT NAME	COMPONENT #	STORAGE	SHELF LIFE
ArciTect™ Human B2M sgRNA	200-0126	Store at -80°C. Alternatively, store at -20°C for up to 6 months.	Stable for 12 months from date of manufacture (MFG) on label.
ArciTect™ Cas9 Nuclease	76002	Store at -20°C.	Stable for 3 years from date of manufacture (MFG) on label.
Anti-Human Beta-2 Microglobulin Antibody, Clone 35	100-0407 (APC)	Store at 2 - 8°C.	Stable until expiry date (EXP) on label. Protect from prolonged exposure to light. Do not freeze.
	100-0408 (PE)		
	100-0409 (FITC)		

Antibody Properties

Target Antigen Name: B2M
Alternative Names: Beta-2-microglobulin, Beta chain of MHC Class I molecules, Beta-2-microglobin, IMD43
Entrez Gene ID: 567
Species Reactivity: Human
Host Species: Mouse

Clonality: Monoclonal

Clone: 35

Isotype: Mouse IgG1

Immunogen: Recombinant human B2M protein

Conjugate: APC (allophycocyanin), PE (phycoerythrin), or FITC (fluorescein isothiocyanate)

Concentration: 0.1 mg/mL

Formulation: Aqueous solution containing 0.5% bovine serum albumin (BSA) and 0.03% ProClin™ 300

Applications: Flow cytometry

Purification: Antibody was produced from a hybridoma resulting from fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, recombinant human B2M and conjugated with either APC, FITC, or PE under optimal conditions. The solution is free of unconjugated APC, PE, or FITC.

Materials Required But Not Included

- Nuclease-Free Water (Catalog #79001)
- Dulbecco's Phosphate Buffered Saline With 2% Fetal Bovine Serum (PBS + 2% FBS) (Catalog #07905)

Directions for Use

A. PREPARATION OF sgRNA STOCK SOLUTION

1. Centrifuge the vial of ArciTect™ Human B2M sgRNA before opening.
2. Add 20 µL of nuclease-free water to give a final concentration of 100 µM. Mix thoroughly.

   NOTE: If not used immediately, aliquot and store at -20°C for up to 6 months. Alternatively, store at -80°C for long-term storage. After thawing aliquots, use immediately. Do not re-freeze.

B. GENOME EDITING OF CELLS WITH sgRNA

1. Prepare RNP Complex Mix by combining ArciTect™ Cas9 Nuclease (4 µg/µL or 25 µM) with sgRNA stock solution (prepared in section A) 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 medium). For both electroporation and chemical transfection methods, a 1:2 - 1:8 molar ratio of Cas9:sgRNA is recommended. RNP complex formation must be optimized for cell type and transfection method.
2. Incubate RNP Complex Mix at room temperature (15 - 25°C) for 10 - 20 minutes.
3. Deliver RNP Complex Mix into cells using your preferred transfection method.
4. Add cells to cultureware and incubate at 37°C for 48 - 72 hours to allow genome editing to occur.

C. ANALYSIS OF EDITING EFFICIENCY BY FLOW CYTOMETRY

For more information on general flow cytometry tips and setup, refer to the "Flow Cytometry Considerations" section of the Technical Bulletin: Flow Cytometry Methods for Identifying Mouse Hematopoietic Stem and Progenitor Cells (Document #27103), available at www.stemcell.com or contact us to request a copy.

1. Collect cells using an appropriate dissociation reagent (e.g. ACCUTASE™, Catalog #07920). Centrifuge at 300 x *g* for 5 minutes.
2. Remove supernatant and resuspend cells in appropriate medium (e.g. D-PBS [Without Ca++ and Mg++], Catalog #37350). Perform a cell count.
3. Per condition, transfer 5 x 10^4 - 1 x 10^5 cells to each tube or well of an appropriate vessel for the flow cytometer to be used, e.g. Corning® 96-Well Round-Bottom Microplate (Catalog #38018) or Falcon® Round-Bottom Polystyrene or Polypropylene Tubes (Catalog #38007 or 38057). In addition, prepare an unlabeled sample, single-antibody labeled sample(s), and fluorescence-minus-one (FMO) "gating" controls. For genome editing experiments, non-electroporated or mock electroporated samples are typically used for these controls.

   NOTE: Extra cells can continue to be cultured, or collected for genomic analyses. For example, genomic DNA can be isolated using the Genomic DNA Purification Kit (Catalog #79020), followed by target-specific amplification using primers flanking the target region with ArciTect™ High-Fidelity DNA Polymerase Kit (Catalog #76026), and sequencing of the PCR products. Alternatively, ArciTect™ T7 Endonuclease I Kit (Catalog #76021) can be used to assess editing efficiency (% INDEL formation) following PCR amplification.

4. Centrifuge at 300 x *g* for 5 minutes. Remove supernatant.
5. Resuspend cell pellet in 100 µL PBS + 2% FBS. Add 2 µL fluorophore-conjugated Anti-Human Beta-2 Microglobulin Antibody. Incubate at room temperature in the dark for 10 - 15 minutes.

   NOTE: Additional stains and/or antibodies (e.g. viability dye, antibodies against cell type-specific markers, etc.) can be added here for combinatorial labeling.
6. Centrifuge at 300 x *g* for 5 minutes. Remove supernatant.
7. Wash cell pellet with 100 - 200 µL PBS + 2% FBS. Centrifuge at 300 x *g* for 5 minutes. Remove supernatant.
8. Resuspend cell pellet in 100 - 200 µL PBS + 2% FBS. Analyze by flow cytometry.

Data

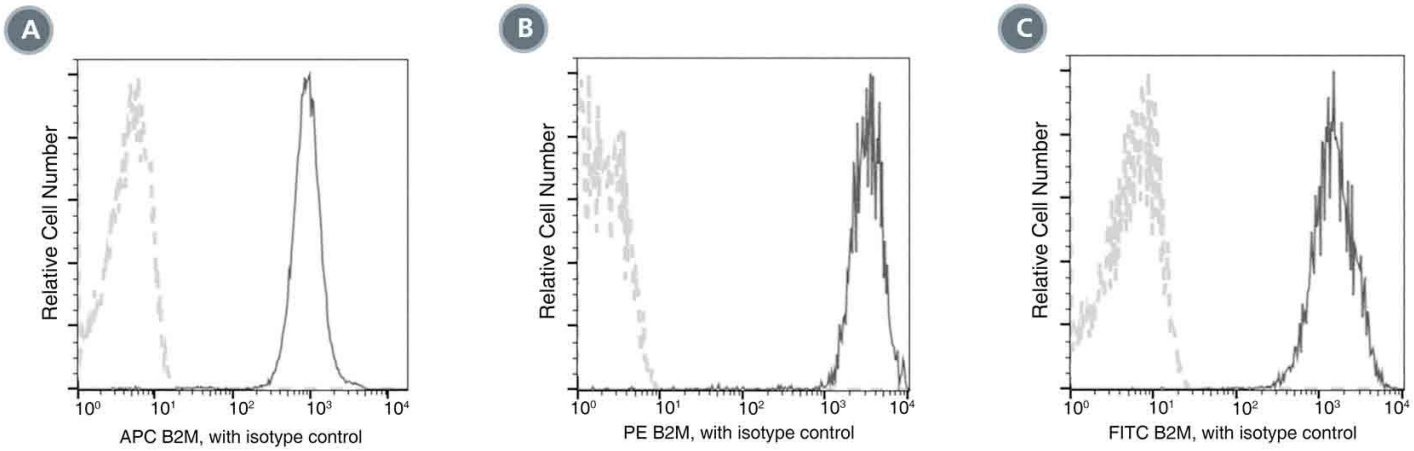

Figure 1. Flow cytometric analysis of human whole blood lymphocytes with Anti-Human Beta-2 Microglobulin Antibody, Clone 35, **(A)** APC-conjugated, **(B)** PE-conjugated, and **(C)** FITC-conjugated. Cells were labeled with fluorophore-conjugated Anti-Human Beta-2 Microglobulin Antibody (solid line histogram) or isotype controls (dashed line histogram). Histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes.

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.