Product Specification Sheet

Product Name	Cre Recombinase Reporter HEK293 Cell Line
Description	The Cre/LoxP recombination system is a versatile and powerful tool for DNA manipulations such as deletions, insertions, translocations and inversions at specific sites in the DNA of cells. The system uses Cre recombinase to recombines a pair of *Lox* sites. Depending on the direction and distance of the LoxP sites, DNA flanked by the LoxP sites can be deleted, inversed, or translocated. Cre Recombinase Reporter HEK293 Cell Line was derived from HEK293 cells by transducing lentiviral particles that carry a DNA fragment in which two loxP sites in the same direction are included. Located between the two loxP sites are the neomycin expression cassette consisting of EF1 promoter, neomycin resistance gene, and bGH poly(A) signal. This cell line stably expresses neomycin-resistant gene and therefore is resistant to neomycin. This cell line will be very useful as a reporter to detect Cre recombinase activity.
Catalog #	CRE01
Size	1x10^6 cells/vial
Shipping	Dry Ice
Storage and Stability	Store in vapor phase of liquid nitrogen immediately upon receipt. This product is stable for 6 months when stored as directed.
Quality Control	Each vial contains approximately 1x10^6 cells with >95% viability before freezing. Each lot of cells are tested for Cre activity, growth, and viability following recovery from cryopreservation, and free of mycoplasma and competent lentivirus as well.
Safety Precautions	**Alstem highly recommends that protective gloves, a lab coat, and a full-face mask are always worn when handling frozen vials.** It is important to note that some liquid nitrogen can leak into the vials when submersed in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in excessive pressure within the vial that can cause the vial to explode or expel the cap with dangerous force.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Overview

The Cre/LoxP recombination system is a versatile and powerful tool for DNA manipulations such as deletions, insertions, translocations, and inversions at specific sites in the DNA of cells. The system uses Cre recombinase to recombines a pair of Lox sites. Depending on the direction and distance of the LoxP sites, DNA flanked by the LoxP sites can be deleted, inversed, or translocated.

Cre Recombinase Reporter HEK293 Cell Line was derived from HEK293 cells by transducing a lentivirus plasmid that carries a DNA fragment in which two loxP sites in the same direction are included. Located between the two loxP sites are the neomycin expression cassette consisting of EF1 promoter, neomycin resistance gene, and bGH poly (A) signal (**Figure 1A**). This cell line stably expresses neomycin and therefore is resistant to neomycin. This cell line will be very useful as a sensitive reporter for detecting Cre recombinase activity. When cells were transfected with a Cre expression plasmid (pLenti-EF1-Cre-GFP-PGK-PURO, Cat. Number: CRE02), DNA fragment in between the two loxP sites was deleted in the stable cell line, but not in non-transgenic cells or wild type cells (**Figure 1 B-C, Figure 2**).

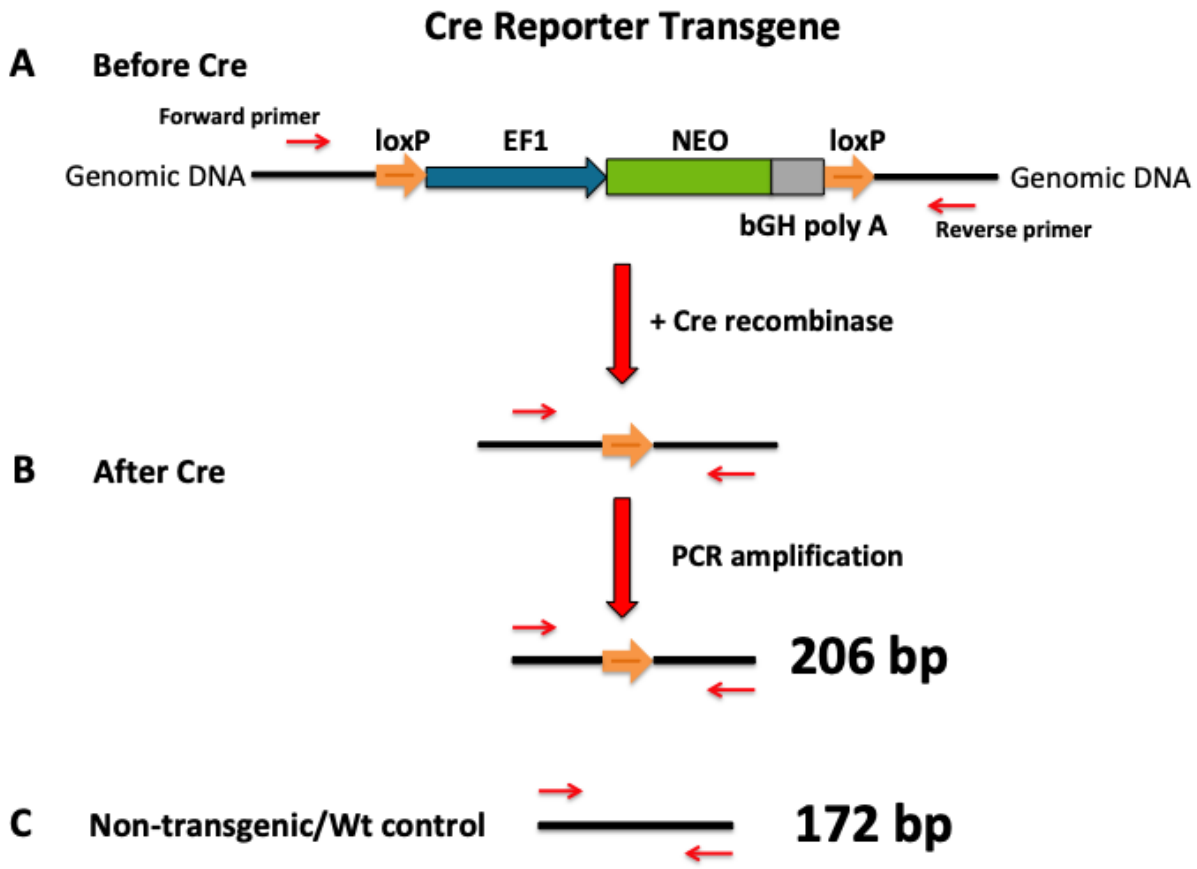

Figure 1. Cre Reporter Stable Cell Line Panel **A**) Before addition of Cre recombinase. The stable cell line carries the transgene that contains a human genomic DNA with an insertion flanked by loxP sites. Panel **B**). After addition of the Cre recombinase, the insertion is deleted. This event is detected by PCR amplification of a 206 bp fragment. Panel **C**) The same PCR amplifies a shorter (172 bp) fragment in the non-transgenic and wild type DNA.

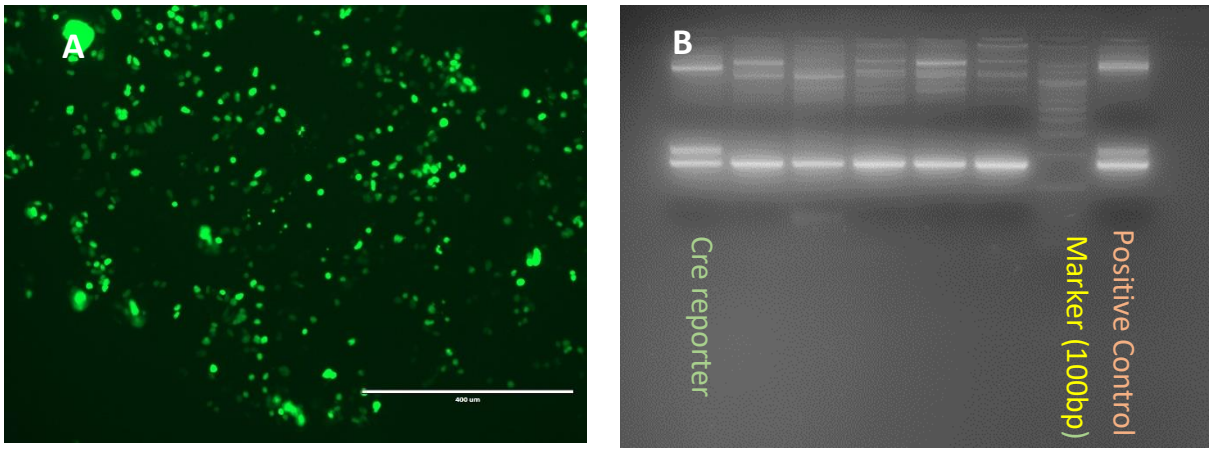

Figure 2. Cre activity detection in Cre reporter cell line. Panel **A**) GFP fluorescence. HEK293 cells were transfected with a Cre-GFP plasmid at 80% cell confluency and express Cre-GFP as green signals. Panel **B**) PCR results. Cre reporter cell line shows efficient DNA deletion as revealed by a larger PCR fragment that contains a loxP site in addition to the human genomic DNA. Positive control template: Cre reporter plasmid. Expected bands: DNA removal = 206 bp, Wild type DNA = 172 bp.

Protocol

Culture Medium:

90% DMEM with GlutaMAX™-I (high glucose) (Thermo Fisher, Cat# 10569-010),

+ 10% Fetal bovine serum (FBS) (Gibco, Cat# 26400-036)

+ 1% Penn/Strep (Thermo Fisher, Cat# 15140-122)

Thawing Cells

1. Add 5 mL of fresh culture medium into a T25 flask.
2. Place the flask in a humidified 37 °C / 5% CO2 incubator for 15 min to allow medium to equilibrate to the proper pH and temperature.
3. Take the vial of cells from liquid nitrogen and rapidly thaw by placing at 37 °C in a water bath with gentle agitation until half thawed. (About 1–2 min, do not submerge vial in water.)
4. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
5. Transfer the vial contents to a sterile 15-mL conical tube. Add 10 mL of warm culture medium drop-wise into the tube, gently mixing as the medium is added.
6. Centrifuge cells at 200 × g for 5 min at room temperature.
7. Aspirate supernatant and resuspend the cell pellet in 1 mL of fresh culture medium.
8. Transfer contents to the T-25 flask containing pre-equilibrated culture medium and place flask in a humidified 37 °C / 5% CO_2 incubator.
9. Change culture medium every other day or when pH decreases. Monitor the cells every day.

Maintenance of Cells

1. Cells were split when they reach about 85% confluence.
2. Pre-warm fresh culture medium in fresh culture vessel in the incubator.
3. Aspirate medium from growing cells, rinse once with PBS, add 0.05% Trypsin/EDTA (2 mL for a T-25 flask, 5 mL for a T-75 flask, 10 mL for a T-175 flask) and swirl to evenly cover the cells. Incubate for 3min. After 3 min, tap the flask firmly on the side. Cells usually detach easily.
4. Add an equal volume of culture medium (10 mL for a T-175 flask) to inactivate Trypsin/EDTA and break up the cell clumps by gently pipetting up and down several times.
5. Verify under a microscope that cells have dislodged and clumps have completely dispersed.
6. Count the cell number using a hemocytometer. Dilute cells 1:1 with 0.4% trypan blue. Add 10ul to a hemocytometer and count the cells. Count at least 4 quadrants.
7. Centrifuge cells at 200 × g for 5 min at room temperature.
8. Aspirate supernatant and resuspend the cell pellet in 1 mL of fresh culture medium.
9. Plate 2x10^6 cells per T-175 flask.
10. Media volume for each T-175 flask would be 20ml.
11. Incubate at a humidified 37 °C / 5% CO_2 incubator.
12. Change media every other day, until the cells reach 85% confluence. Monitor the cells every day. The cells should be 60-70% confluent, three days after plating.

Freezing Cells

1. Aspirate the medium and wash the cells twice with 20 ml of warm DPBS per T-175 flask.
2. Aspirate DPBS, and add 10 ml of 0.05% trypsin-EDTA per T-175 flask, and incubate for 2-3 min. After 3 min, tap the flask firmly on the side. The cells should be observed coming off the flask easily.
3. Add 10 ml of culture medium and break up the cell clumps by gently pipetting up and down several times.
4. Transfer the cells to a 50 mL conical tube per T-175 flask. Count the number of cells using 0.4% trypan blue.
5. Centrifuge at 200 g for 5 min at room temperature
6. Aspirate the supernatant, resuspend the pellet in cold EZStem™ Freezing Media (Cat# M050) at 1x10^6 cells/ml.
7. Transfer 1 mL of cell suspension in freezing medium into each labeled cryogenic vial.
8. Place the vials in a Mr. Frosty cell-freezing container and keep it at -80°C overnight.
9. Transfer the vials to a liquid nitrogen tank for long-term storage.

IMPORTANT NOTICE

Store the vials at vapor phase of liquid nitrogen immediately upon receipt.

WARNING

Do not use cryogenic vials for storage in the liquid phase of liquid nitrogen. Such use may cause entrapment of liquid nitrogen inside the vial and lead to pressure buildup resulting in possible explosion or biohazard release. Use appropriate safety procedures that are outlined by the ATCC when handling and disposing of vials. ALSTEM highly recommends that protective gloves and clothing be used and a full face mask always be worn when handling frozen vials.