CRISPR Activation Plasmid Transfection

PROTOCOL

Santa Cruz Biotechnology, Inc.

PHASE 1: CRISPR Activation Plasmid Transient Transfection

This protocol is recommended for a single well from a 6-well tissue culture plate. Adjust cell and reagent amounts proportionately for wells or dishes of different sizes.

- In a 6-well tissue culture plate seed 1.5 x 10^5 - 2.5 x 10^5 cells in 3 ml of antibiotic-free standard growth medium per well, 24 hours prior to transfection. Grow cells to a 40–80% confluency. Initial cell seeding and cell confluency after 24 hours are determined based on the rate of cell growth of the cells used for transfection. Healthy and subconfluent cells are required for successful KO and HDR Plasmid transfection.

NOTE: The optimal Plasmid DNA:**UltraCruz® Transfection Reagent** (sc-395739) ratio should be determined experimentally beginning with 1 µg of Plasmid DNA and between 5–15 µl of UltraCruz® Transfection Reagent. Once the Transfection Reagent volume is optimized to minimize cell toxicity, Plasmid DNA concentrations can vary between 1–3 µg per well. If the optimal UltraCruz® Transfection Reagent volume is 10 µl, then Plasmid DNA concentrations ranging from 1–3 µg/10 µl should be tested. For example, test Plasmid DNA/UltraCruz® Transfection Reagent amounts: 1 µg/10 µl, 2 µg/10 µl, and 3 µg/ 10 µl. The appropriate amount of Plasmid DNA/UltraCruz® Transfection Reagent complex used per well should be tested to determine which amount provides the highest level of transfection efficiency.

- Prepare the following solutions:

  Solution A: For each transfection, dilute 1–3 µg of Plasmid DNA into **Plasmid Transfection Medium** (sc-108062) to bring final volume to 150 µl. Pipette up and down to mix. Let stand for 5 minutes at room temperature.

  Solution B: For each transfection, dilute 5–15 µl of **UltraCruz® Transfection Reagent** (sc-395739) with enough **Plasmid Transfection Medium** (sc-108062) to bring final volume to 150 µl. Pipette up and down to mix. Let stand for 5 minutes at room temperature.

NOTE: Do not add antibiotics to the **Plasmid Transfection Medium** (sc-108062).

- Add the Plasmid DNA solution (Solution A) dropwise directly to the dilute UltraCruz® Transfection Reagent (Solution B) using a pipette. Vortex immediately and incubate for no less than 20 minutes at room temperature.
- Prior to transfection, replace media with fresh antibiotic-free growth medium. Add the 300 µl Plasmid DNA/UltraCruz® Transfection Reagent Complex (Solution A + Solution B) dropwise to well.
- Gently mix by swirling the plate.
- Incubate the cells for 24–72 hours under conditions normally used to culture the cells. No media replacement is necessary during the first 24 hours post-transfection. Add or replace media as needed 24–72 hours post-transfection.

PHASE 2: Cell Selection

- Select stable activated clones via **Puromycin dihydrochloride** (sc-108071), **Hygromycin B** (sc-29067) and **Blasticidin S HCl** (sc-495389) selection. For antibiotic selection, use an amount sufficient to kill the non-transduced cells. Puromycin concentrations ranging from 2-10 µg/ml, Hygromycin B concentrations ranging from 200-500 µg/ml and Blasticidin S HCl concentrations ranging from 1-20 µg/ml are usually sufficient, but a selective antibiotic titration is recommended for every cell line or cell type used.
- Replace medium with fresh selective antibiotic-containing medium every 3-4 days, until resistant colonies can be identified. Pick several colonies, expand them and assay them for stable target gene activation.

NOTE: Resulting selective antibiotic-resistant clones may have varying levels of target gene activation due to the random integration of the Activation constructs into the genome of the cell.

PHASE 3: Cell Assay

- For cells transfected with CRISPR Activation Plasmid, assay cells 48-72 hours after transfection step.
- For protein analysis, change media to standard growth medium 3 days prior to cell lysis. To lyse adherent cells, aspirate media, rinse cells with PBS, scrape and centrifuge cells at low speed to obtain a cell pellet. For suspension cells, transfer the culture to a centrifuge tube and centrifuge cells at low speed to obtain a cell pellet. Wash once with PBS and centrifuge again. For 100% confluent HEK 293 or HeLa cells, add 100 µl of **RIPA Lysis Buffer System** (sc-24948) to the pellet. For other cell lines or confluencies, the amount of RIPA Lysis Buffer System to use should be determined experimentally. Sonicate or shear cells. Incubate sample on ice for 10 minutes, vortex, and incubate again for 10 minutes on ice. Spin cell lysate at 10000 RPM for 20 minutes at 4° C. Use the BCA Protein Assay Kit: sc-202389 to determine protein concentration.
- For RT-PCR analysis isolate RNA using the method described by P. Chomczynski and N. Sacchi (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162: 156-159) or a commercially available RNA isolation kit.

References:
PMID: 24157548, PMID: 23287718

RECOMMENDED SUPPORT PRODUCTS

PRODUCT	CAT. #	DESCRIPTION	AMOUNT
UltraCruz® Transfection Reagent	sc-395739	Delivers CRISPR Activation Plasmid into cells with minimal cell toxicity; enables highly efficient DNA transfection in a variety of cell lines including HeLa, A549, Jurkat and NIH/3T3.	0.2 ml
Plasmid Transfection Medium	sc-108062	Reduced-serum medium suitable for addition to CRISPR Activation Plasmid and Plasmid Transfection Reagent immediately prior to cell transfection; modification of Eagle's Minimal medium, buffered with HEPES and sodium bicarbonate, and supplemented hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, growth factors and phenol red.	20 ml
Control CRISPR Activation Plasmid	sc-437275	Control Scrambled gRNA CRISPR Activation Plasmid is a negative control for experiments using a target-specific CRISPR Activation Plasmid; encodes a non-targeting scrambled gRNA that does not recognize any DNA sequence. The SAM complex will not bind DNA nor activate transcription of any specific gene.	20 µg

CRISPR Activation Plasmid support products are optimal for successful delivery of Santa Cruz Biotechnology, Inc.'s CRISPR Activation Plasmid into mammalian cells. Amounts listed above are based on use of 6-well plates.