I. General Considerations

A. Storage

- Store Cre Recombinase Gesicles (Cat. No. 631449) at –70°C upon receipt.
- After use, return the unused portion of Cre Recombinase Gesicles to –70°C.
- Cre Recombinase Gesicles can be freeze-thawed up to ten times.

B. Stability

Cre Recombinase Gesicles are stable for one year from date of receipt under proper storage conditions.

C. Additional Materials Required

The following materials are required but not supplied:

- Complete medium without antibiotics
- Polybrene (Hexadimethrine bromide; Sigma, Cat. No. H9268)

D. Cell Plating & Cre Delivery Conditions

Plate your cells one day prior to treatment. They should be 60–80% confluent on the day of treatment. Cultures that are less than 50% confluent at the time of Cre delivery may lose viability.

NOTES:

- Upon first use, we recommend using a range of Cre Recombinase Gesicle volumes in order to maximize the recombination efficiency for your specific cell type.
- Cre Recombinase Gesicles also contain CherryPicker™ protein, a membrane-targeted version of the mCherry fluorescent protein that helps to confirm delivery of the Cre recombinase to your target cells.

II. Procedure: Treatment of Target Cells with Cre Recombinase Gesicles

This protocol describes an application using adherent cells cultured in 12-well plates. Suspension cell cultures should be treated at their recommended growth density. Perform the following steps under sterile conditions:

1. Check that cells plated on the previous day are about 60–80% confluent.
2. Prepare Polybrene (PB) media by supplementing complete medium with 6 µg/ml Polybrene.
3. Replace the complete media with 1 ml PB media.
4. Thaw Cre Recombinase Gesicles at room temperature and keep on ice.
5. Add 10 µl of Cre Recombinase Gesicles per well.
   Return the unused portion of Cre Recombinase Gesicles to –80°C.
6. Centrifuge the 12-well plate at 2,500 rpm for 15–30 min at room temperature.
   NOTE: Centrifugation increases delivery efficiency. In the event that centrifugation cannot be performed, add more Gesicles and increase incubation time to as long as overnight.
7. Incubate the cells for at least 2 hr at 37°C in an appropriate cell culture incubator.
8. After incubation, replace PB media with fresh media without PB and continue culturing the cells.
9. Assay cells as required.
   NOTE: Cre Recombinase Gesicles contain CherryPicker protein, a membrane-targeted version of the mCherry fluorescent protein that helps to confirm delivery of the Cre recombinase to your target cells. Fluorescence will decrease over time (~48–72 hr) as the mCherry protein is degraded.

Contact Us For Assistance	
Customer Service/Ordering	**Technical Support**
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This document has been reviewed and approved by the Clontech Quality Assurance Department.