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Technical Data Sheet: Raji-GFP-Luc2

ATCC® Number	CCL-86-GFP-LUC2™
Organism	*Homo sapiens*
Tissue/Disease Source	Burkitt's lymphoma
Product Description	This cell line was derived from Raji (ATCC® CCL-86™) cells by transduction with a lentiviral vector containing both the green fluorescent protein (GFP) and firefly luciferase (Luc2) genes and subsequently through single cell cloning. • Signal-to-noise ratio: > 100 • Naturally expresses high levels of CD19 (verified at ATCC) • Used as target cancer cells for in vitro killing assays by CD19 CAR-T cells (tested at ATCC) and is expected to also work for CD20 CAR-T cells.
Application	Excellent signal/background ratio and stable luciferase and GFP expression. It can be used in CAR-T cytotoxicity assays or cell-based assays for cancer research.

CAR-T Cytotoxicity Assay

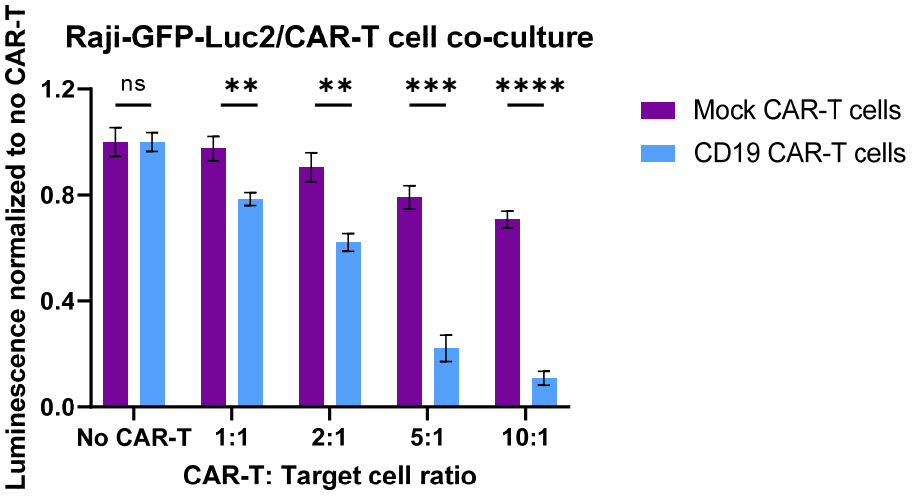

Figure 1: CD19 CAR-T cell in vitro killing assay of Raji-GFP-Luc2 cells measured using luminescence. 5 x 10^3 Raji-GFP-Luc2 cells were co-cultured with either mock (purple) or CD19 (blue) CAR-T cells (ProMab) derived from the same donor. Co-cultures were performed at varying CAR-T cell to target cell ratios (1:1, 2:1, 5:1 or 10:1). After 24 hours of co-culture, Bright-Glo reagent (Promega) was added and luminescence was determined using a plate reader. Luminescence values were normalized to no CAR-T wells. Error bars indicate the standard deviation of three biological replicates. ns = not significant, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$, unpaired t-test.

GFP Expression

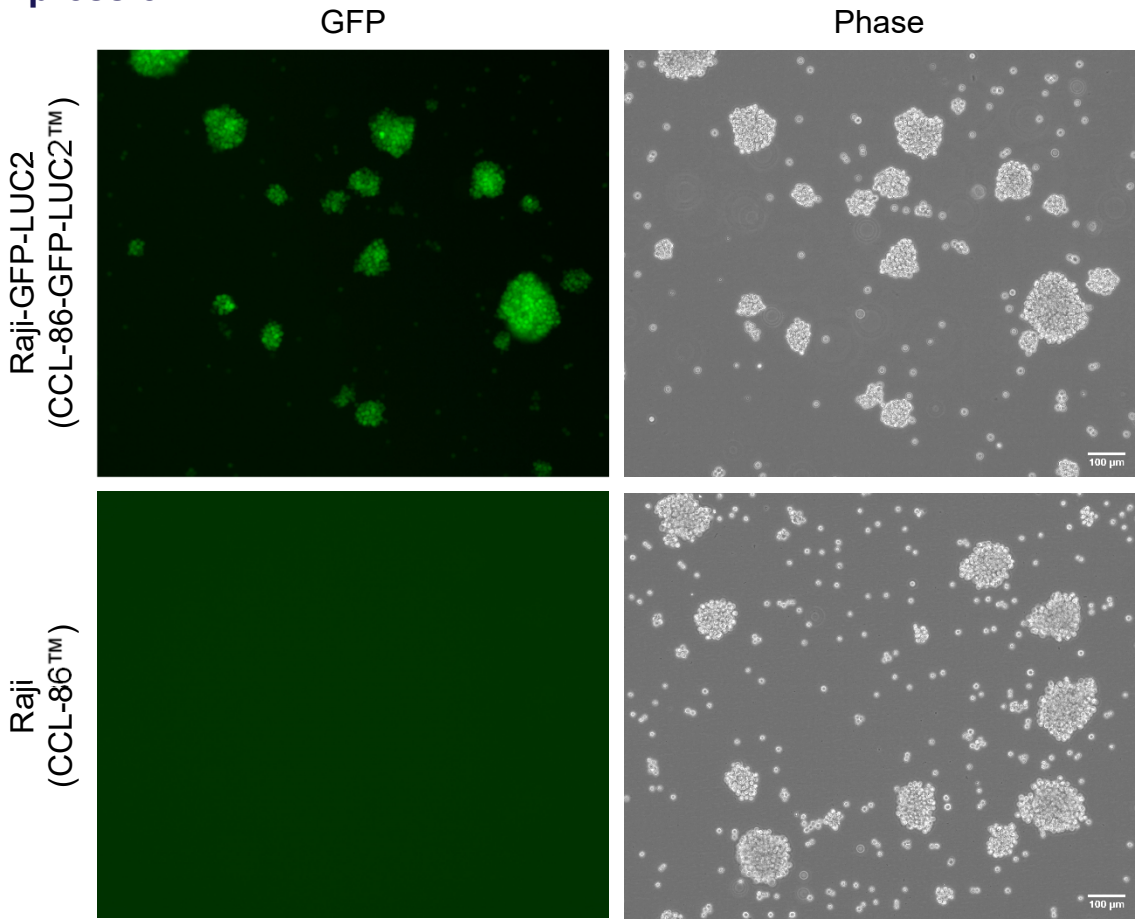

Figure 2: Cell morphology and GFP expression of Raji-GFP-Luc2 cells. Raji-GFP-Luc2 (top) and Raji (bottom) cells were seeded at 1 x 10^5 cells/ml and GFP (left) and phase contrast (right) images were captured three days later. Cells were maintained in ATCC-recommended culture conditions. Doubling times were determined to be 20.2 hr for Raji-GFP-Luc2 and 19.8 hr for Raji. Scale bars, 100 µm.

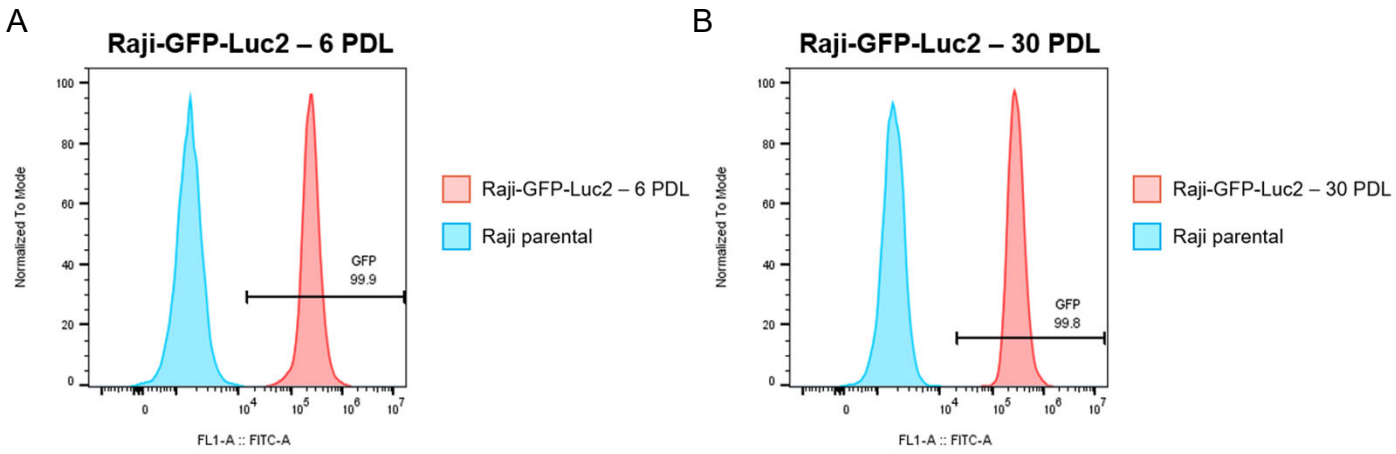

Figure 3: GFP expression in Raji-GFP-Luc2 cells is stable after 30 population doublings. GFP expression was detected by flow cytometry using 10,000 cells per sample for Raji-GFP-Luc2 cells (red) after (A) six population doublings or (B) 30 population doublings. Raji parental cells (blue) were used as a negative control.

Luciferase Expression

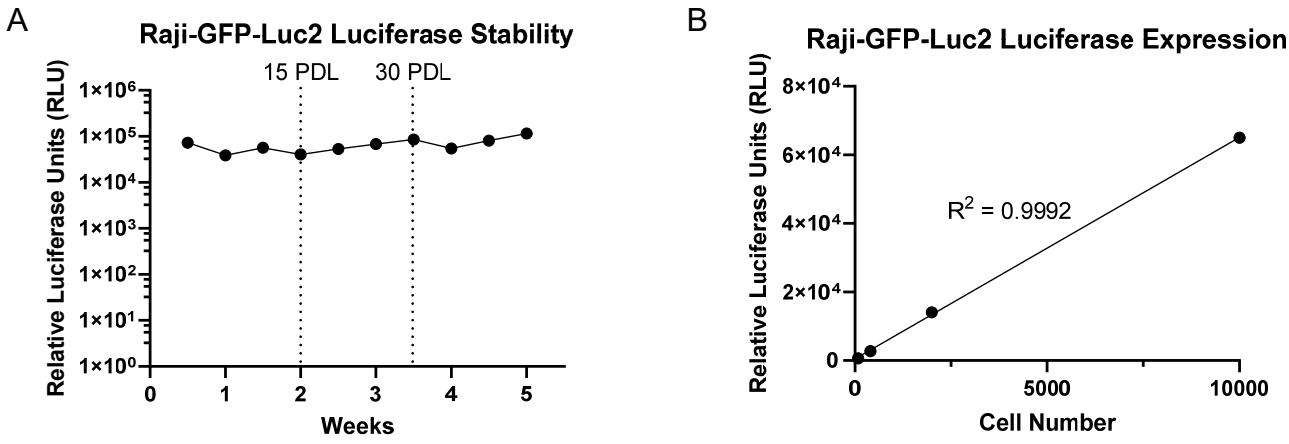

Figure 4: Luciferase stability and linear correlation with cell number in Raji-GFP-Luc2 cells. A) Bright-Glo reagent (Promega) was added to 10,000 Raji-GFP-Luc2 cells in triplicate before every passage over the course of more than 30 population doublings. Luminescence was read using a plate reader within 10 minutes. B) Bright-Glo reagent (Promega) was added to 80, 400, 2,000, or 10,000 Raji-GFP-Luc2 cells in triplicate. Luminescence was read using a plate reader within 10 minutes.

CAR-T Target Expression

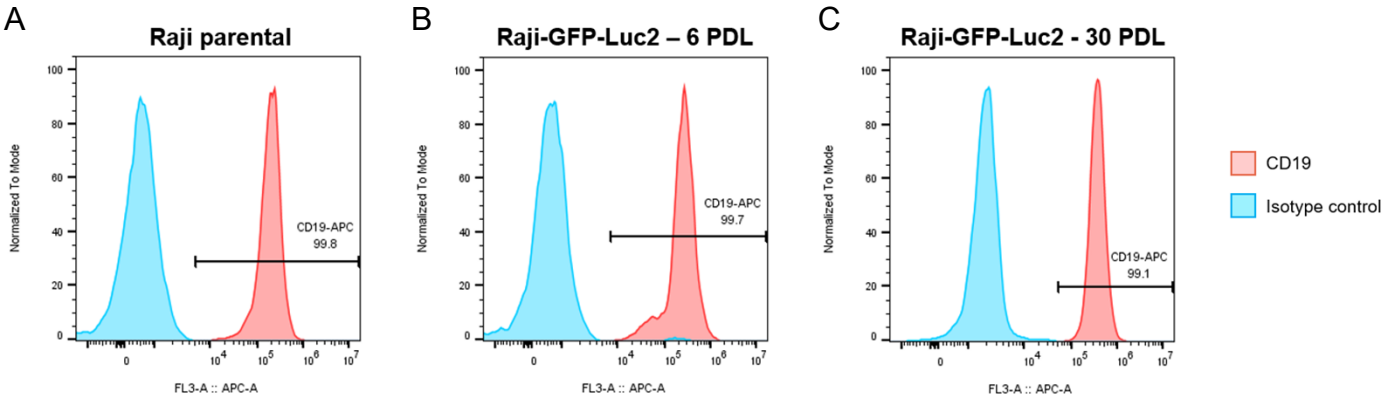

Figure 5: Raji-GFP-Luc2 cells stably express high levels of CD19. CD19 expression was detected by flow cytometry using 10,000 cells per sample for (A) Raji parental, (B) Raji-GFP-Luc2 after six population doublings (center), or (C) Raji-GFP-Luc2 after 30 population doublings (right). APC-conjugated human anti-CD19 antibody (red) (Miltenyi Biotec REA675) or APC-conjugated human IgG1 isotype control (blue) (Miltenyi Biotec REA293) was used at a 1:50 dilution per sample.