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Technical Data Sheet: NCI-H929-GFP-Luc2

ATCC® Number	CRL-3580-GFP-LUC2™
Organism	*Homo sapiens*
Tissue/Disease Source	Plasmacytoma
Product Description	This cell line was derived from NCI-H929 (ATCC® CRL-3580™) 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: > 500 • Naturally expresses high levels of BCMA (tested at ATCC) • Used as target cancer cells for in vitro killing assays by BCMA CAR-T cells (tested at ATCC)
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

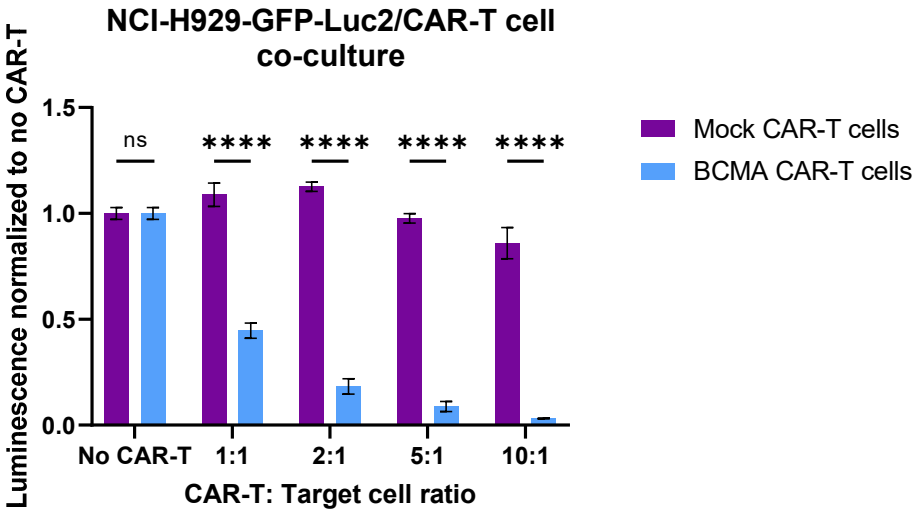

Figure 1: BCMA CAR-T cell in vitro killing assay of NCI-H929-GFP-Luc2 cells measured using luminescence. 5 x 10^3 NCI-H929-GFP-Luc2 cells were co-cultured with either mock (purple) or BCMA (blue) CAR-T cells (ProMab) derived from the same donor. Co-cultures were performed at varying CAR-T cell to target 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, **** = $p < 0.0001$, unpaired t-test.

GFP Expression

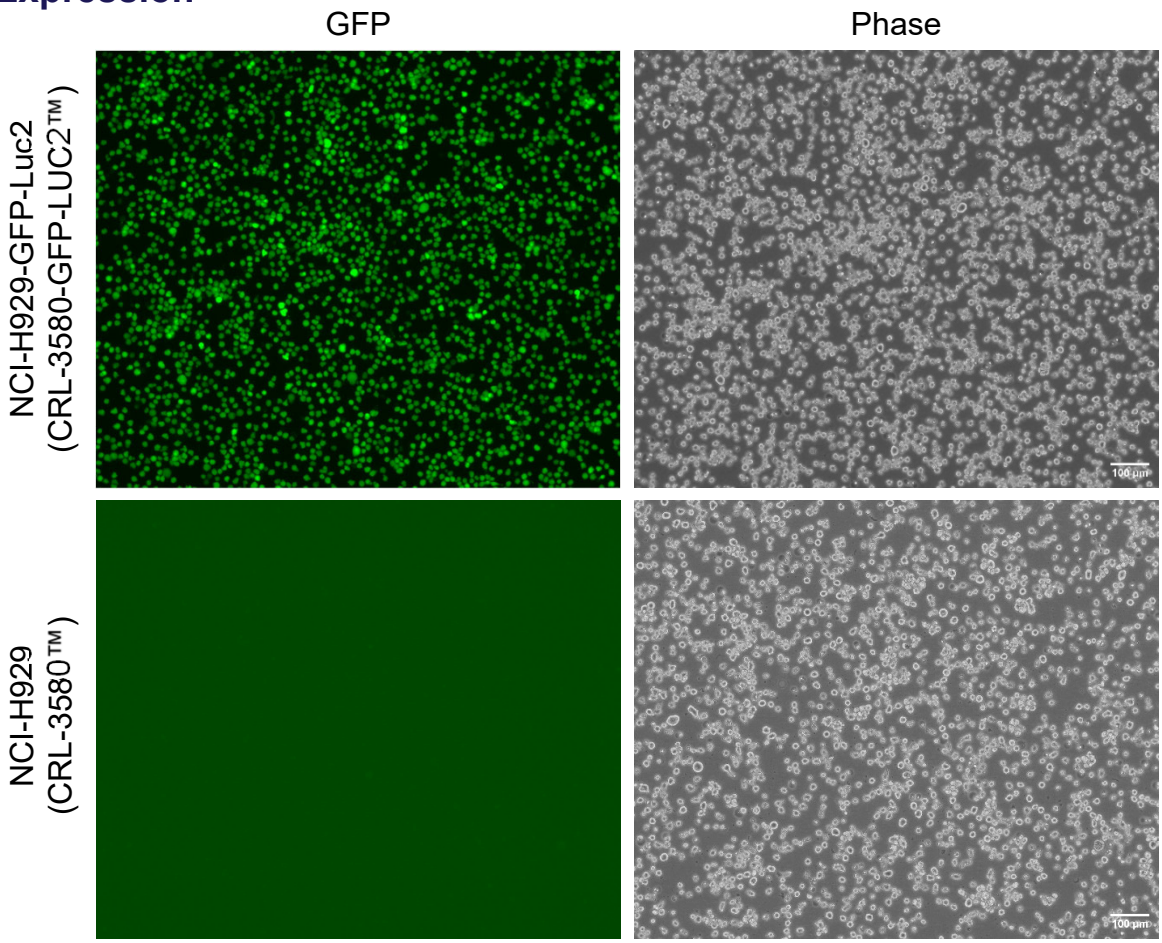

Figure 2: Cell morphology and GFP expression of NCI-H929-GFP-Luc2 cells. NCI-H929-GFP-Luc2 (top) and NCI-H929 (bottom) cells were seeded at 3 x 10^5 cells/ml and GFP (left) and phase contrast (right) images were captured three days later. Cells were maintained in ATCC-recommended culture conditions. Doubling times were determined to be 28.6 hr for NCI-H929-GFP-Luc2 and 29.6 hr for NCI-H929. Scale bars, 100 µm.

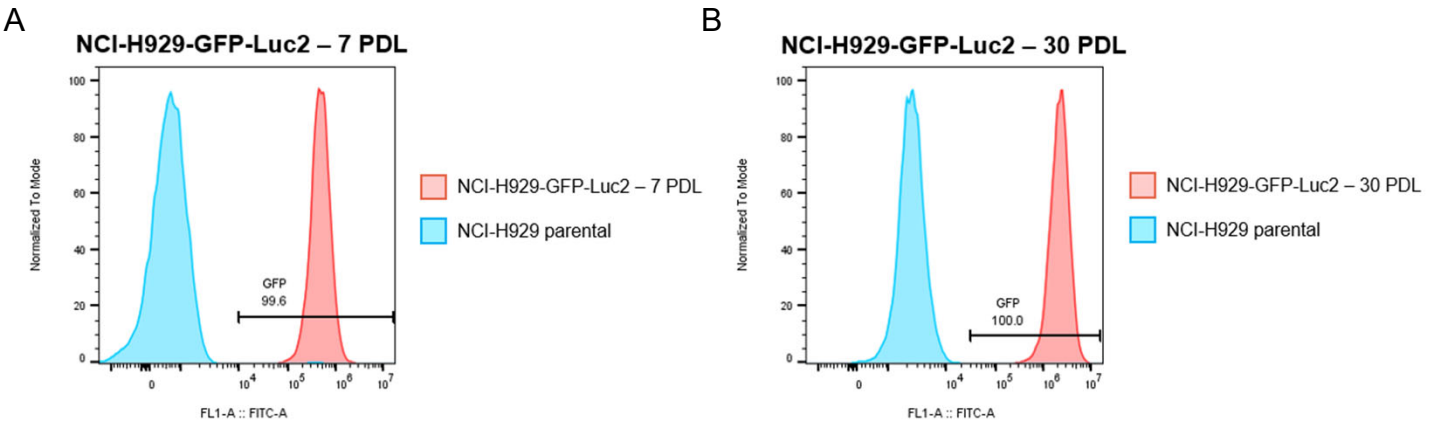

Figure 3: GFP expression in NCI-H929-GFP-Luc2 cells is stable after 30 population doublings. GFP expression was detected by flow cytometry using 10,000 cells per sample for NCI-H929-GFP-Luc2 cells (red) after (A) seven population doublings or (B) 30 population doublings. NCI-H929 parental cells (blue) were used as a negative control.

Luciferase Expression

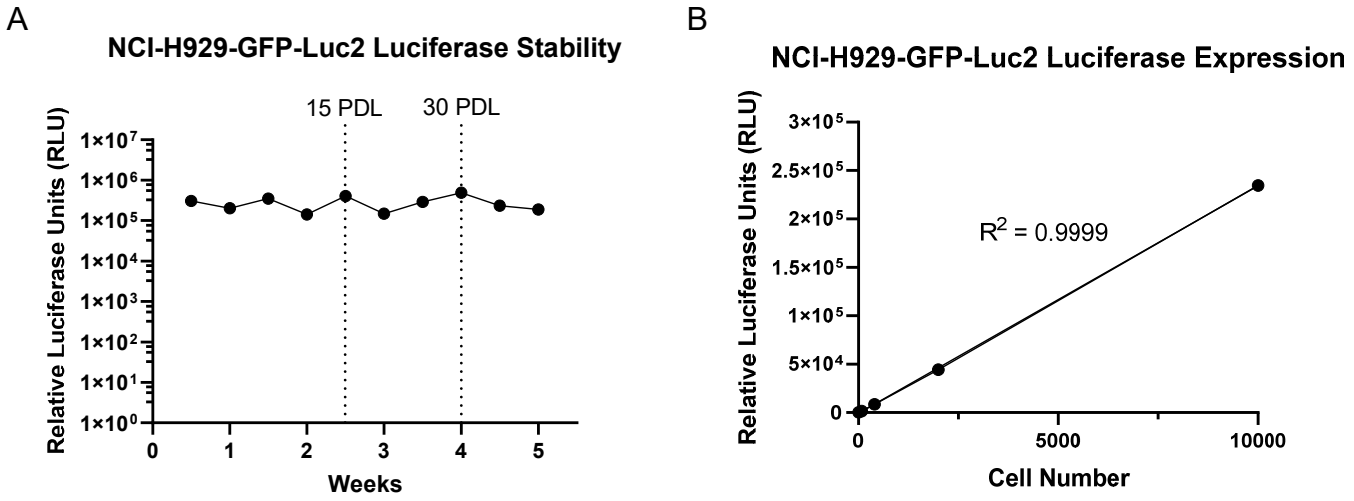

Figure 4: Luciferase stability and linear correlation with cell number in NCI-H929-GFP-Luc2 cells. A) Bright-Glo reagent (Promega) was added to 10,000 NCI-H929-GFP-Luc2 cells in triplicate before every passage over the course of more than 30 population doublings. Luminescence was read using a plate reader within 10 minutes. B) Bright-Glo reagent (Promega) was added to 80, 400, 2,000, or 10,000 NCI-H929-GFP-Luc2 cells in triplicate. Luminescence was read using a plate reader within 10 minutes.

CAR-T Target Expression

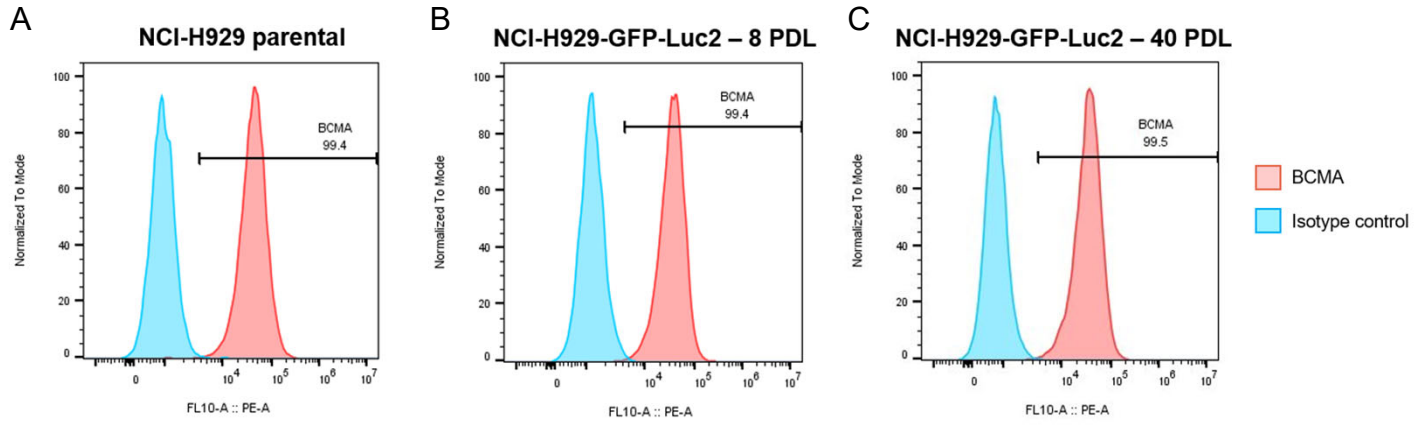

Figure 5: NCI-H929-GFP-Luc2 cells stably express high levels of BCMA. BCMA expression was detected by flow cytometry using 10,000 cells per sample for (A) NCI-H929 parental, (B) NCI-H929-GFP-Luc2 after eight population doublings, or (C) NCI-H929-GFP-Luc2 after 40 population doublings. 5 µg of PE-conjugated mouse anti-BCMA antibody (BD Pharmingen 570547) or 5µgof PE-conjugated mouse IgG1, κ isotype control (BD Pharmingen 554680) was used per sample.