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Technical Data Sheet: BT-474-Luc2

ATCC® Number	HTB-20-LUC2™
Organism	*Homo sapiens*
Tissue/Disease Source	Ductal carcinoma
Product Description	This luciferase expressing cell line was derived from BT-474 cell line by transduction with lentiviral vector encoding firefly luciferase gene (luc2) and subsequently through single cell cloning. • Signal noise ratio: ≥ 1,000 • Bioluminescence: ≥ 100,000 photons/cell/sec (subject to imaging and culture condition) • Naturally expresses high levels of HER2 (verified at ATCC) • BT-474-Luc2 has been used as a target cancer cell for in vitro killing assay by HER2 CAR-T cells (tested at ATCC)
Application	Excellent signal/background ratio and stable luciferase expression make this cell line ideal for in vitro study of HER2 specific CAR-T cells. It also can be used in cell-based assays for cancer research and in vivo bioluminescence imaging of xenograft animal model to study human cancer and monitor activity of anti-cancer drug

In vitro CAR-T killing cancer bioluminescence assay

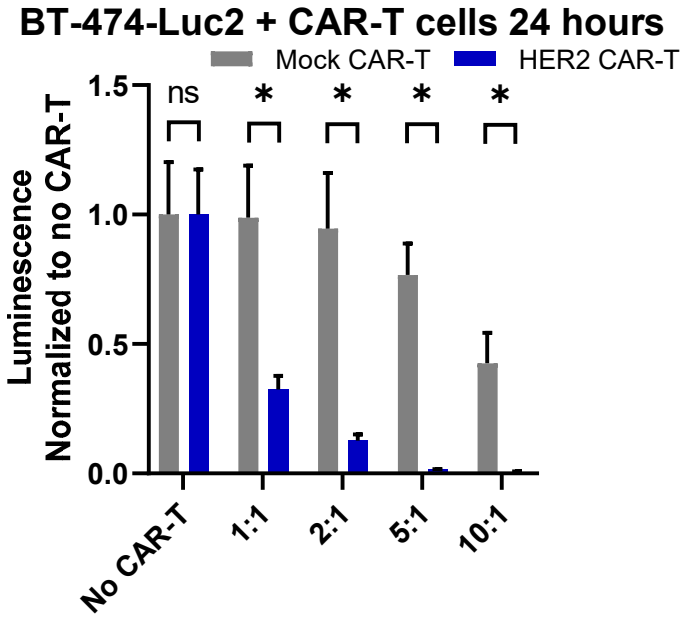
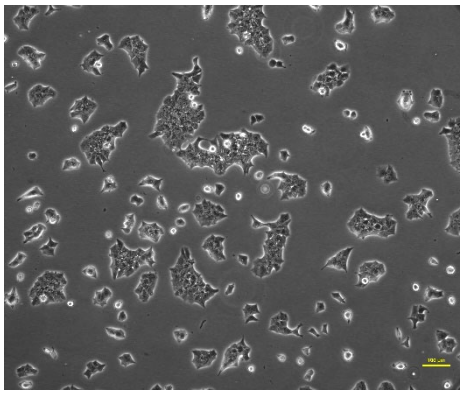


Figure 1: HER2 CAR-T in vitro killing assay of BT-474-Luc2 measured using luminescence. BT-474-Luc2 cells (5 x 10^3) were seeded into a 96-well plate and were used as target cells for either HER2 CAR-T or Mock CAR-T (control) from the same donor which were seeded at various ratios of CAR-T cells to target BT-474-Luc2 cells (1:1, 2:1, 5:1, and 10:1). After 24 hours of co-culture, Bright-Glo (Promega) was added to the indicated wells. The luminescence of the plate was read within 10 minutes using a luminescence plate reader and determined to have a dose-dependent specific killing with HER2 CAR-T cells which was greater than the non-specific killing observed with mock CAR-T cells. (* = significant difference, ns = not signicigant using unpaired t test, with a single pooled variance).

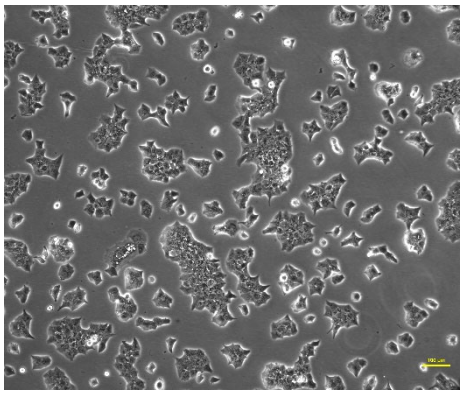
Cell Morphology

BT-474 (HTB-20™)



Doubling time = 59.2 hours

BT-474-Luc2 (HTB-20-LUC2™)



Doubling time = 60.8 hours

Figure 2: Cell morphology of BT-474 parental and BT-474-Luc2. Cells were maintained in ATCC recommended culture conditions. Cell morphology was observed under microscopy and images were captured by digital camera.

In vivo Bioluminescent Imaging

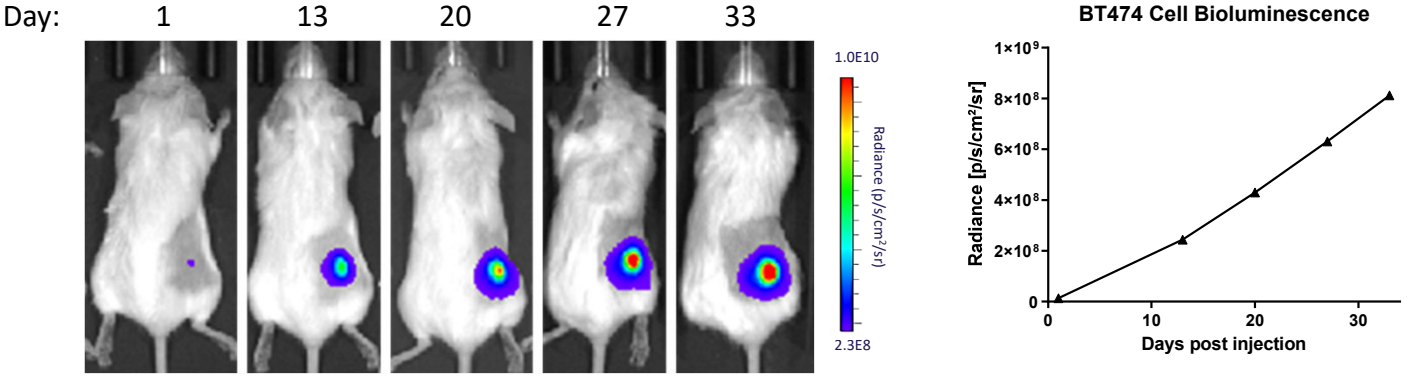


Figure 3: In vivo detection of luciferase activity of BT474-Luc2. BT-474-Luc2 cells ($2.8x10^6$) were injected subcutaneously into the dorsal region near the thigh of female NSG mice. Tumor growth was monitored weekly using a Xenogen IVIS Spectrum. *In vivo* bioluminescence imaging demonstrated the progression of tumors.

Luciferase Expression

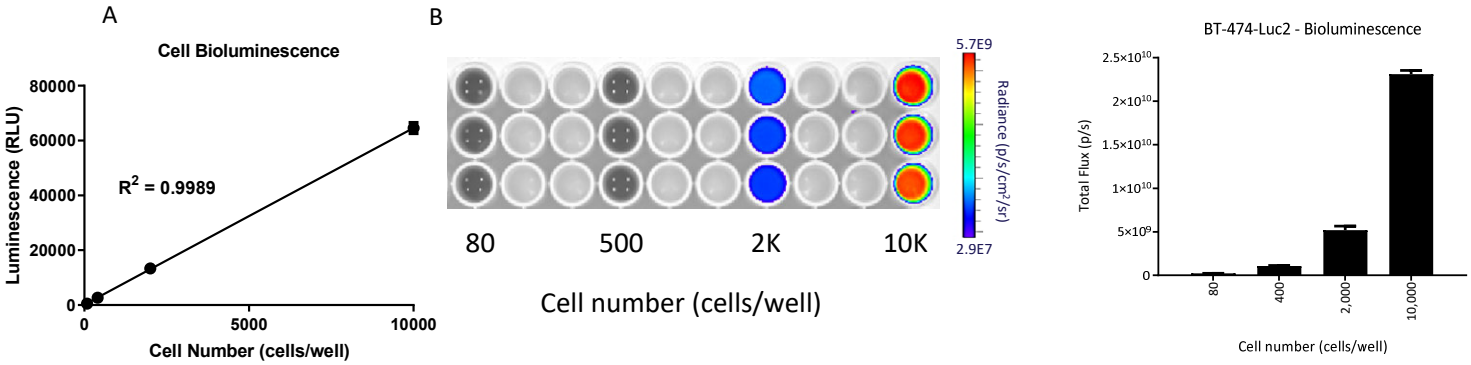


Figure 4: Linearity of luminescence and of in vitro quantification of luciferase activity of BT-474-Luc2. Cells were seeded in a 96-well plate at indicated cell numbers per well, and Bright-Glo (Promega) was added to the indicated wells. The luminescence of the plate was read within 10 minutes using a luminescence plate reader (A) and determined to have a linear correlation of bioluminescence intensity with cell numbers. (B) The plate was imaged using a Xenogen IVIS Spectrum to quantify that photons emitted per cell.