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Technical Data Sheet:
BT-474 ECAD-EmGFP EMT Reporter Cell Line

ATCC® Number	**HTB-20 EMT™**
Organism	*Homo sapiens*
Tissue/Disease Source	Breast; ductal carcinoma
Product Description	Here, we created an ECAD-EmGFP reporter cell line (HTB-20EMT) using the CRISPR/Cas9 gene editing platform and the parental BT-474 breast ductal carcinoma cell line (HTB-20). This cell line harbors a C-terminal green fluorescent protein (EmGFP) tag on the E-cadherin gene, enabling the tracking of the EMT status of cells *in vitro* by monitoring GFP expression. The integrity of the ECAD-EmGFP knock-in has been verified at the genomic, mRNA and protein level for sequence and expression.
Application	The BT-474 ECAD-EmGFP reporter cell line provides a convenient and sensitive platform for research on the mechanisms of metastasis *in vitro* and the development of new antitumor drugs for metastatic breast cancer.

E-cadherin (ECAD) Reporter Expression

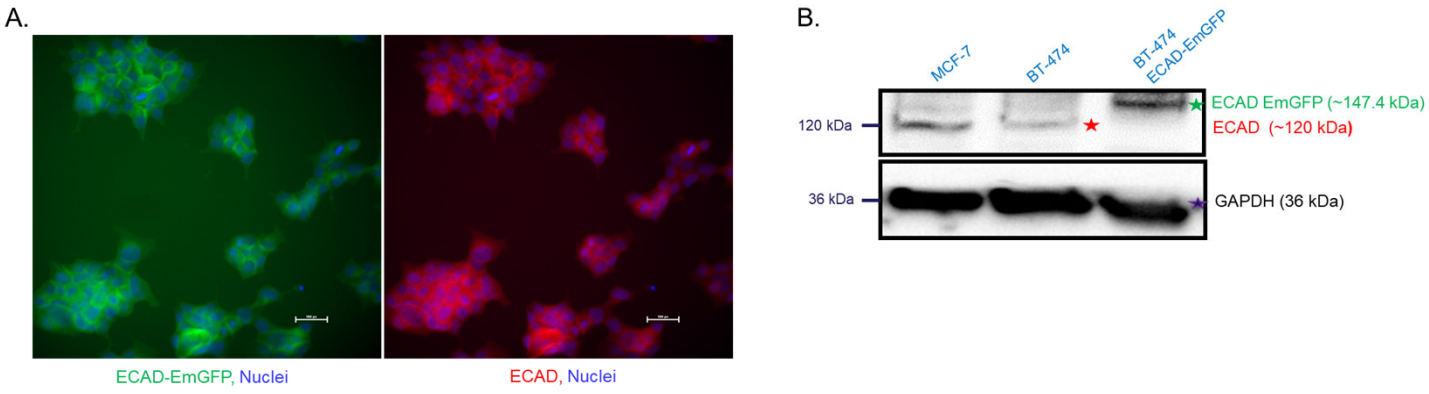

Figure 1. EmGFP activities faithfully report ECAD gene expression in BT-474 ECAD-EmGFP cells. (A) ECAD protein detected by immunofluorescence analysis (right, red) is co-localized with EmGFP (left, green). (B) To verify the ECAD-EmGFP knock-in allele and the expression of ECAD-EmGFP fusion protein, BT-474 parental cells, BT-474 ECAD-EmGFP cells, and MCF-7 cells were subjected to Western blot. An ECAD antibody detected an approx. 120 kDa. wild type ECAD protein in BT-474 parental cells, and an approx. 147 kDa. ECAD-EmGFP fusion protein in ECAD-EmGFP cells. MCF-7, an epithelial cell line expressing a high level of ECAD, was used as a positive control. GAPDH was used as a loading control..

EMT Induction

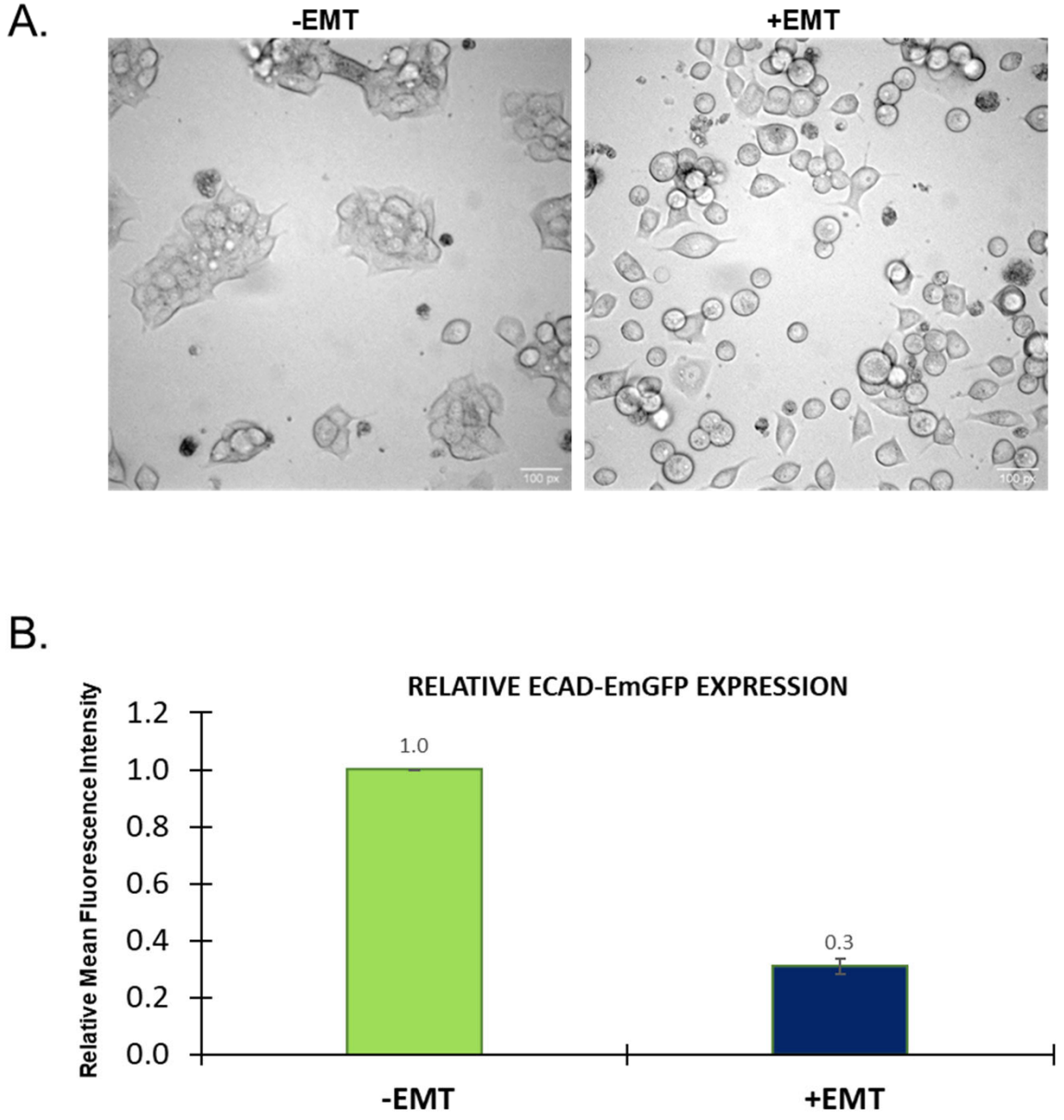

Figure 2. BT-474 ECAD-EmGFP cells undergo EMT upon incubation with StemXVivo inducing agent. BT-474 ECAD-EmGFP cells were incubated in DMEM medium containing 10% FBS and supplemented with either 2X StemXVivo (R&D system, cat# CCM017) EMT inducing media supplement (+ EMT) or an equivalent volume of 1X DPBS (as a no EMT control; - EMT) for 5 days. (A) Morphology of "+ EMT" BT-474 ECAD-EmGFP cells exhibited dramatic changes upon induction compared with that of the "- EMT" control. (B) Treatment of BT-474 ECAD-EmGFP cells with 2X StemXVivo EMT inducing media supplement resulted in a significant decrease in ECAD-EmGFP expression.

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