PROTOCOL TO MEASURE TACE ACTIVITY (ADAM10 / ADAM17):

Published in: Nandhu et al., *Cancer Res.* (2014) 74(19): 5435-5448. Novel paracrine modulation of Notch-DLL4 signaling by fibulin-3 promotes angiogenesis in high-grade gliomas.

Reagent: TACE peptide substrate type III, R&D systems (# ES003). Stock is 4mM (8 mg/ml) in DMSO

Positive control: Purified mouse ADAM17, R&D systems (# 2978-AD). Dissolve at 100 ug/ml in milliQ water

Assay buffer:
50 mM Tricine
100 mM NaCl
10 mM $CaCl_2$
1 mM $ZnCl_2$
pH 7.5

Assay for acute inhibition or activation of TACE activity:

a) SAMPLES

1. Incubate cells with vehicle (PBS) or treatment for 30-60min
2. Wash cells with cold PBS
3. Collect cells in assay buffer containing 0.1 %v/v Triton X100
4. Vortex cells well (do not sonicate) and keep on ice for 10min
5. Centrifuge at 2,500 g x 10min at 4C. If desired, save pellet at -80C
6. Keep supernatants on ice while measuring total protein per sample. Use equal amounts of total protein to measure TACE activity

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b) SUBSTRATE

1. Prepare TACE substrate by diluting 1/200 of stock substrate in assay buffer (concentration at this point will be 20 uM). Prepare the substrate only at this point.
2. Make enough substrate to add 50 ul to each well in the assay below.

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c) ASSAY

1. Prepare the following samples in a 96-well plate (all samples in triplicate). Use a special plate with black walls for fluorescence detection.

Tests: Protein sample: 20 to 100 ug. Complete with assay buffer to 50 ul

Negative control: Assay buffer 50 ul

Positive control: Purified TACE: 50 to 100 ng. Complete with assay buffer to 50 ul

2. Add 50 ul of the diluted TACE substrate to each well. Cover with aluminum foil
3. Wait 5 min at room temperature and then start measuring fluorescence in a fluorimeter plate reader (excitation: 320 nm / emission: 405 nm)
4. Continue measuring fluorescence every 10 min for a total of 6h or until the values reach a plateau.
5. Calculate fluorescence (units) per mg protein for each treatment and compare treated versus control cells at each time point.

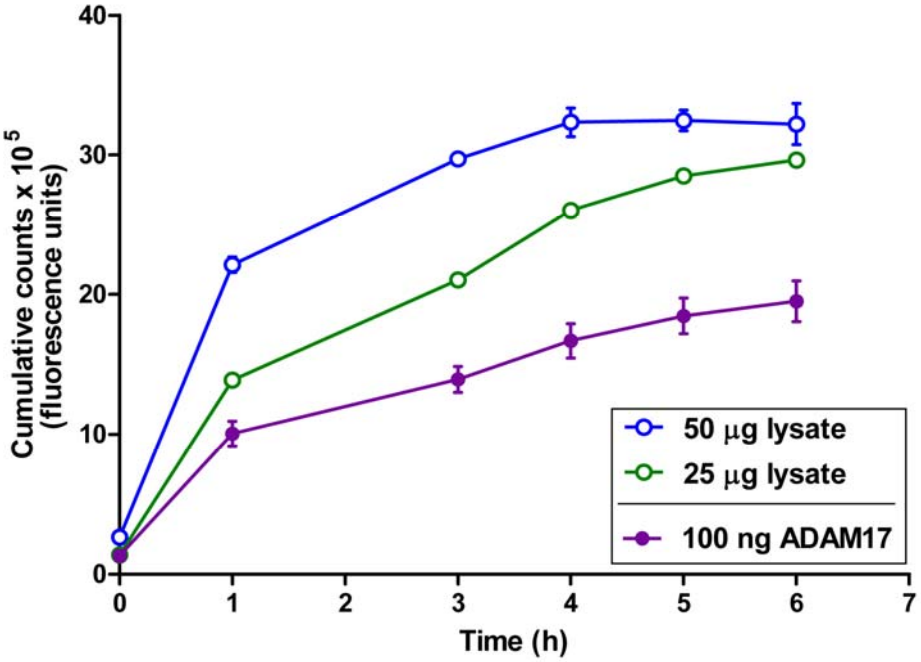

Figure: Lysate of rat CNS1 glioma cells prepared as indicated in the protocol above and compared against purified ADAM17