Neprilysin Activity Assay

Substrate DAGNPG (N-dansyl-Ala-Gly-D-nitro-Phe-Gly, modeled after enkephalins with an aromatic moiety in the P'1 position and a short residue in the P'2 position) is an internally quenched substrate of NEP. NEP degrades DAGNPG to DAG (Km = 45 μM, V = 0.65 μmol/mg protein/min), and the liberated dansyl group can be excited at a wavelength of 342 nm and emits at 562 nm. ACE can also degrade DAGNPG, therefore an ACE inhibitor is necessary to resolve NEP activity (must pre-incubate for 10 min, using at least 0.5 μM captopril). NEP has optimal activity in 50 mM Tris HCl, pH 7.4, and inhibited by 20 nM Thiorphan (Ki = 3 nM).

Prepare a stock 25 mM DAGNPG solution in methanol (store at 4ºC).

1. Prepare 100μg lysate in 50 mM Tris HCl, pH 7.4 with captopril. The final volume (per reaction, including substrate) should be 200 μL.

2. Make a 1 mM DAGNPG solution in Tris. Add DAGNPG to the lysate for a 50 μM final concentration, mix and incubate at 37ºC for desired time points.

3. Stop the reaction by heating to 100ºC for 5 min. Store tubes on ice until all time points are reached.

4. Spin 5,000g x 5 min at RT.

5. Remove 175 μL sample and dilute into 400 μL Tris. Vortex to mix and add 200 μL to a 96-well plate.

4. Read on the Victor2 multilabel plate reader (excitation, 355; emission, 550, optimal wavelengths are 342/562).