ACE Activity Assay

Angiotensin-converting enzyme (ACE) activity can be measured using the substrate hippuryl-L-histidyl-L-leucine (HHL). ACE cleaves the substrate to expose a free N-terminus, which can be fluorogenically labeled with *o*-phthaldialdehyde (OPA).

HHL Sigma #H4884 FW=429.5
Prepare a 50mg/ml stock solution (116.4mM) in acetic acid. Store -20º for up to 1 year.
OPA Sigma #P1378 FW=134.1
Prepare a 20mg/ml stock solution in Ethanol. Store +4ºC.
Use a final amount of 1mg per 100µl reaction

NaB (Sodium Borate) Buffer -final concentration is 0.4M Boric Acid, 0.3M NaCl, pH 8.3. Prepare a 2x stock solution.

Protocol:
Use 2.5 µg lysate and 1mM HHL in a 35µl final volume of 1x NaB buffer.

1. Preincubate the enzyme with any inhibitors (the ACE inhibitor captopril, for example) for 20 min in a 25µl volume in 1x buffer (no substrate).

2. Prepare 3.5mM HHL substrate in 1x NaB buffer.
Neutralize HHL by adding approximately 1.9 times the HHL volume of 10M NaOH. Adjust pH to 8.3 (proper pH is critical for ACE activity).

3. Mix the 25µl of prepared enzyme from Step 1 with 10µl of 3.5mM HHL for a 35ul total volume. Incubate 37ºC for desired timepoints (10-60 min, depending on activity of sample).

4. Stop reaction by adding 150ul 0.34M NaOH, invert to mix.

5. Add 20µl 20mg/ml OPA. Invert to mix and incubate 10 min at RT.

6. Stop reaction by adding 50ul 3M HCl

8. Read on a fluorescent plate reader at 355nm excitation; 535nm emission.