E2 (ARD) Activity assay:

1. Make stock solutions:

E1buffer: 50 mM Hepes, 0.5 mM MgCl₂, pH 7.5. (Filter, degas, Ar bubble).

2. Get:

Ice
E1sub, E1, catalase (store on ice).

3. Procedure:

990 µl E1 buffer (under stream of Ar)
5 µl Catalase (20 mg/ml)
5 µl E1
1 µl Substrate
1000 µl Total

Monitor A₃₀₅.

Open to air, add 2 µl of enzyme to be tested.

Monitor A₃₀₅ to make sure the reaction goes to completion.

4. Test 98S8A with “ITC7A”, “no E”, “Ni only” controls.
5. Use the above reaction mixtures to assay for α-keto acid and formate.