

BMN TEST REACTION

The test reaction requires considerable patience and diligence to perform, but provides a good test to verify the mixing and quenching times. It is not advised as a method to troubleshoot a KinTek quench-flow since the instrument has been fully verified. Consult the factory for methods to test for leaks or other needed repairs. Because the quantitation of the reaction depends upon an absorbance measurement, you must be careful to recover 100% of the sample and the total volume must be constant.

RATIONALE: The base-catalyzed hydrolysis of benzylidene malanonitrile (BMN) occurs with a second order rate constant of $140 \text{ M}^{-1}\text{s}^{-1}$, so the reaction with 1 M NaOH gives a rate of 140 s^{-1} , and a lower concentration could be used to get a slower rate if desired. The kinetics of hydrolysis can be monitored at 310 nm continuously in a stopped-flow and compared to samples mixed and quenched in a quench-flow instrument. The reaction is begun by mixing with NaOH and terminated by mixing with HCl. BMN is only moderately stable at low pH, so the absorbance measurements need to be made within an hour of quenching.

STOCK SOLUTIONS

1 mM BMN: Dissolve approximately 2 mg of BMN in 3.0 ml isopropanol. Dilute to 1 mM BMN with 5 mM HCl just prior to use.

4 N HCl

2 N NaOH

4 M Potassium Acetate: The pH of this solution will be approximately 6.5 and does not need to be adjusted.

PROCEDURE

1. **Check the sample recoveries in each loop.** Place 5 mM HCl in the Quench syringe, then load 1 mM BMN in 5 mM HCl into one sample loop and 5 mM HCl into the other. Collect samples in duplicate for each of the sample loops. Be sure the collection tubes have the appropriate additional volume of quench solution (5 mM HCl) to make sure that the volume of each quenched sample is the same. Add 600 μl of H₂O to each sample and then read the absorbance at 310 nm.
2. **React BMN with 2 N NaOH** (1 M final after mixing) in the quench-flow. Place 4 N HCl in the quench syringe. Prepare to collect time points from 2-40 msec. Add the appropriate extra volume of quench solution to each tube to maintain a constant volume.
3. After collecting each sample, add a fixed volume (70 μl) of 4 M K Acetate to bring the final pH to about 4.5. Add 600 μl of H₂O to each sample and then read the absorbance at 310 nm.
4. There should be no variability in the recovery of the BMN with various loops, but if there is, normalize the results from each sample loop relative to the absorbance of the sample recovered in the blank run in step 1 of this procedure. Alternatively, by increasing the step distance for each loop by 200 steps, you can achieve closer to 100% recovery. The reaction should follow a single exponential with a rate of 140 sec⁻¹ at 20°C when reacting with 1 M NaOH.