

β-GALACTOSIDASE REFOLDING ASSAY

Materials/Reagents

1M Glycylglycine (pH 7.2)

β-galactosidase

Unfolding buffer (25mM HEPES pH 7.4, 5mM MgCl₂, 50mM KCl, 5mM β-mercaptoethanol, and 6M guanidine-HCl)

Refolding buffer (25mM HEPES pH 7.4, 5mM MgCl₂, 50mM KCl, 2mM ATP, 10mM DTT)

Proteins:

BSA

Hsp70

Hdj1

Other desired proteins

ONPG (0.8M)

Sodium carbonate (0.5M)

Procedure:

Preparation

- 1) Prepare 30°C and 37°C water baths.
- 2) Add 10μL of 10μg/μL β-galactosidase to 90μL 1M Glycylglycine (pH 7.2).
- 3) Add 5μL of the above mixture to 95μL Glycylglycine (native) and to 95μL of Unfolding buffer.
- 4) Incubate at 30°C for 30 minutes.

Prepare reaction tubes:

- 1) 124μL total volume of refolding buffer with:
- 2) Positive/Negative controls:
 - 3.2μM BSA
- 3) Experiments
 - 1.6μM Hsp70
 - 3.2μM Hdj1
 - 1.6μM Hip
 - 1.6μM Bag1
 - Other proteins

Prepare substrate:

- 1) Add 10μL 0.8M ONPG to separate tubes for each reaction corresponding to time points of 0, 30, 60, 90, 120, 180, and 240 minutes.

- 2) Add 80 μ L of 0.5M sodium carbonate to all 0 time points except the BSA positive and negative controls.

Running the experiment:

- 1) Once incubation of β -galactosidase is done, add 1 μ L Native β -galactosidase to the positive BSA reaction tube, mix immediately by pipetting, take a 10 μ L sample and add it to the BSA positive control time 0 tube containing the ONPG, and put both the reaction tube and the substrate tube in the 37°C water bath leaving the reaction tube lid open.
- 2) Start the timer counting up.
- 3) Add 1 μ L of denatured β -galactosidase to the BSA negative control reaction tube, mix immediately by pipetting, take a 10 μ L sample and put it into the time 0 negative control BSA substrate tube, and put both in the 37°C water bath leaving the reaction tube lid open.
- 4) Continue this process using 1 μ L denatured β -galactosidase for all the test reaction tubes.
- 5) After 15 minutes of incubation of substrate and reaction sample, take it out of the water bath and immediately add 80 μ L 0.5M sodium carbonate to stop the reaction. (Note: only the BSA samples will have this step for the first time 0 point).
- 6) Continue to take 10 μ L samples of the reaction tubes and add it to the corresponding substrate tubes at corresponding time points, incubate at 37°C for 15 minutes, and stop the reaction with 80 μ L of 0.5M sodium carbonate.

Taking data:

- 1) Measure the absorbance of all substrate reaction tubes once the experiment is done at 413nm wavelength.
- 2) Percent activity is calculated by the formula: $100 * (\text{test-absorbance} / \text{native absorbance})$
- 3) Percent activity is graphed verses time.

Troubleshooting/Critical parameters:

The refolding buffer is crucial in the refolding experiment. It should be made precisely as indicated, as accurately as possible. The same aliquot of buffer should not be used more than 2 times as ATP degradation negatively affects the assay.

Pipetting should be done quickly, putting only the tip of the pipette tip into the liquids. Getting extra unfolding buffer into the reaction tubes will prevent refolding from occurring.

References:

Freeman, B.C., and Morimoto, R.I. The human cytosolic molecular chaperones hsp90, hsp70 (hsc70) and hdj-1 have distinct roles in recognition of a non-native protein and protein refolding. *EMBO* **15**:2969-2979 (1996).

Submitted by:

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