Anti-Invertase Assay

Project: Sequencing
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Aim:

To verify the action of anti-invertase on invertase, and determine the concentration needed to inhibit invertase activity.

Materials Required:

- 96 well plate
- 100 ml conical flask
- LB media
- Cloning kit
- Glucose stock
- Sucrose stock
- Pipettes
- 10 µl microtips
- 100 µl microtips

Procedure:

- Clone anti-invertase-histag gene into E.coli K-12 using pET28-a backbone as directed by the Cloning protocol, and plates are prepared (Kanamycin)
- Prepare culture using LB media as directed by the LB Medium Preparation protocol
- Anti-invertase protein is extracted as directed by the [His-tag Antibody protein purification] protocol and stored at -20 °C. (1 L culture is expected to give around 2-3 mg, 4 mg at best, while a 10 L culture can give 25 mg.)
- A commercial **invertase assay kit** employing yeast invertase (Cat.MAK118, Sigma Aldrich, USA) is used as per the manufacturer's protocol to examine the potency of recombinant ShINH1. https://www.sigmaaldrich.com/catalog/product/sigma/mak118?lang=en&region=IN
- 40 µl of reaction volume containing different concentrations of ShINH1, from 0.1 µM to 0.5 µM, in increments of 0.1 µM are pre-incubated in a 96-well plate with commercial acid invertase at 37°C for 30 min.
- Glucose standards (40 µl volume, 0–100 µM glucose) are added to separate wells of the plate. The same volume of reaction buffer was used as the assay blank in separate wells.
- Substrate was added to each well 5 µl of 20 mM sucrose followed by incubation for 20 min at room temperature.
- After incubation, the reaction mixture containing 95 µl of reaction buffer, 1 µl of enzyme mix and 1 µl of dye reagent (all supplied with the kit) was prepared and 90 µl of reaction mixture was added to each of the blank, sample, and standard wells followed by incubation for 20 min at room temperature in darkness.
- The amount of glucose liberated was calculated from the glucose standard curve.
- The specific activity of enzyme was calculated and expressed as µmoles of glucose formed per milligram of protein per minute.

Procedure

	A	B	C	D	E	F	G
1	**Sample**	**[Anti-Invertase Protein] (in µM)**	**Reaction volume of Anti-Inv (in µl)**	**[Sucrose] (in mM)**	**Volume of [Sucrose] stock to be added (in µl)**	**Volume of reaction mix to be added (in µl)**	**Total Volume (in ml)**
2	Blank	0.00	40	20.00	5.00	90.00	0.135
3	1	0.10	40	20.00	5.00	90.00	0.135
4	2	0.20	40	20.00	5.00	90.00	0.135
5	3	0.30	40	20.00	5.00	90.00	0.135
6	4	0.40	40	20.00	5.00	90.00	0.135
7	5	0.50	40	20.00	5.00	90.00	0.135
8	**Total**	1.50	240	120.00	30.00	540.00	0.81