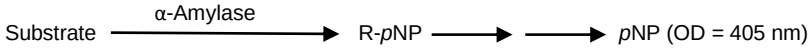
α-Amylase Inhibitor Screening Kit

2/19

(Catalog # K482-100; 100 assays; Store at -20°C)

I. Introduction:

α-Amylases (EC 3.2.1.1) are digestive enzymes which catalyze the hydrolysis of internal α-1,4-glycosidic linkages in starch to lower molecular weight products, such glucose, maltose and maltotriose units. Human α-amylase is mainly expressed in salivary glands and pancreas. These two isozymes share a high degree of primary amino acid sequence similarity (97%) and 92% in their catalytic domains. Furthermore, both α-Amylases are immunologically identical in their reactions with polyclonal antibodies, share same mode of action, preferred substrates, are Chlorine activated and reach maximum activity at similar pH values. Functionally, they have similar but not identical cleavage patterns when tested with a variety of substrates. Modulation of α-amylase activity affects the utilization of carbohydrates as an energy source. This enzyme is responsible for the breakdown of complex carbohydrates in humans. Thus, inhibition of α-amylase could be considered as a strategy for the treatment of disorders related to carbohydrate uptake, such as diabetes, obesity, dental cavities and periodontal diseases. BioVision's α-Amylase Inhibitor Screening Kit provides a simple, homogenous assay for screening potential α-Amylase inhibitors using a 96 well microplate. In our kit, human α-Amylase hydrolyzes the synthetic substrate, yielding smaller fragments containing the chromophore (*p*NP; OD = 405 nm). A potent, specific inhibitor is also included in the kit.



II. Application:

- Screening/characterizing α-Amylase inhibitors.

III. Kit Contents:

Components	K482-100	Cap Code	Part Number
α-Amylase Assay Buffer	55 ml	NM	K482-100-1
α-Amylase Substrate	1 vial	Red	K482-100-2
α-Amylase, Human Saliva	1 vial	Blue	K482-100-3
α-Amylase Inhibitor	100 µl	Orange	K482-100-4

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C. Bring α-Amylase Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **α-Amylase Assay Buffer:** Warm to room temperature before use. Store at 4°C or -20°C.
- **α-Amylase Substrate:** Reconstitute with 100 µl α-Amylase Assay Buffer to prepare stock solution. Aliquot Stock Solution in 10 µl aliquots and store at -20°C. Use aliquot once only.
- **α-Amylase, Human:** Reconstitute with 100 µl α-Amylase Assay Buffer to prepare stock solution. Aliquot Stock Solution in 10 µl aliquots and store at -20°C. Use aliquot once only.
- **α-Amylase Inhibitor:** Ready to use. Keep on ice while in use.

VI. α-Amylase Inhibitor Screening Protocol:

1. **Screening Compounds, Inhibitor Control & Background Control preparations:** Test **Samples [S]** and **Inhibitor Control [IC]:**
   Dissolve test samples 100X in a proper solvent. Further dilute to 3X with α-Amylase Assay Buffer. Mix well.
   Test Samples [S]: Add 50 µl of Diluted test samples (3X) to designated wells of a clear 96 well microplate.
   Inhibitor Control [IC]: Add 10 µl of reconstituted α-Amylase inhibitor to 40 µl Assay Buffer in designated wells.
   Enzyme Control [EC]: Add 50 µl of α-Amylase Assay Buffer to designated wells.
   Background Control [BC]: Add 100 µl of α-Amylase Assay Buffer to designated wells.
   Solvent Control [SC][a]: Add 50 µl of 3X Solvent (the same concentration of solvent as in the diluted Test Samples) in α-Amylase Assay Buffer to designated wells
   IC_{50} estimation (Optional): prepare several dilutions of candidate(s) in α-Amylase Assay Buffer maintaining Solvent Concentration constant for all concentrations. Add 50 µl of each dilution into designated individual wells.

   Notes:

   a. Various organic solvents may reduce the α-Amylase enzymatic activity. Prepare parallel well(s) as **Solvent Control [SC]** to test the effect of the solvent on α-Amylase enzymatic activity. If [SC] slope is significantly different when compared to [EC], use [SC] values to determine effect of the respective tested compounds (see Step 5-Calculations).

2. **α-Amylase Enzyme Solution:** Dilute α-Amylase stock by adding 490 µl of α-Amylase Assay Buffer into 10 µl of α-Amylase Enzyme. Mix thoroughly by pipetting up and down. Add 50 µl of diluted α-Amylase Solution to each well containing Test Sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC] and Solvent Control [SC]. *Avoid introducing any bubbles into the wells.* Gently shake the plate to mix and incubate at room temperature for 10 min. Protect from light.

   Note: Do not store Diluted α-Amylase Enzyme Solution. Discard unused solution.

3. **α-Amylase Substrate Mix Preparation:** Dilute Substrate by adding 490 µl of α-Amylase Assay Buffer into 10 µl of α-Amylase Substrate. Mix thoroughly by pipetting up and down. Add 50 µl of diluted α-Amylase Substrate to each well containing Test Sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC], Background Control [BC] and Solvent Control [SC]. *Avoid introducing any bubbles into the wells*. Mix well.
4. **Measurement:** Measure absorbance at OD = 405 nm in kinetic mode for 20-25 min at room temperature. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).
5. **Calculation:** Subtract the reading of Background Control [BC] from all test samples [S], Enzyme Control [EC], and Solvent Control [SC]. Calculate the slope of all wells by dividing the net ΔOD (OD_2 - OD_1) over time Δt (t_2 - t_1). If [SC] slope is significantly different when compared to [EC], use [SC] values to determine the inhibitory effect of tested compound(s).

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of}[\text{EC}] - \text{Slope of}[\text{S}]}{\text{Slope of}[\text{EC}]} \text{X100}$$

$$\% \text{ Relative Activity} = \frac{\text{Slope of}[\text{S}]}{\text{Slope of}[\text{EC}]} \text{X100}$$

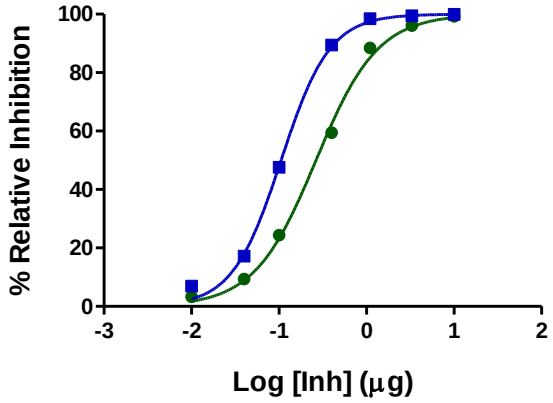


Figure: (**Figure**: Inhibition of α-Amylase, Human Salivary (■) and Pancreas (●) by α-Amylase Inhibitor. IC_{50} of α-Amylase Inhibitor were calculated to be 108± 5 ng and 267 ± 4 ng, respectively. Assay was carried out following the kit protocol.

VII. RELATED PRODUCTS:

Amylase Activity Colorimetric Assay Kit (K711)
Starch Colorimetric/Fluorometric Assay Kit (K647)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
Glycogen Colorimetric/Fluorometric Assay Kit (K646)
Glycogen Colorimetric Assay Kit II (K648)
Maltose Colorimetric/Fluorometric Assay Kit (K628)
Maltose & Glucose Colorimetric/Fluorometric Assay Kit (K618)
α-Amylase Polyclonal Antibody (3014 & 3925)
Rat Pancreatic Amylase Antibody (Clone RPA-B5) (3102)
α-Glucosidase Inhibitor Screening Kit, Colorimetric (K938)
α-Glucosidase Activity Colorimetric Assay Kit (K690)
Glucose Colorimetric/Fluorometric Assay Kit (K666)
Glucose Colorimetric Assay Kit II (K686)
Glucose Uptake Colorimetric Assay Kit (K676)
Glucose Uptake Fluorometric Assay Kit (K666)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
PicoProbe™ Glucose-6-Phosphate Assay Kit (K687)
Total Carbohydrate Assay Kit (K645)

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