

ab197005

Invertase Activity Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Invertase activity in biological samples and purified/crude enzyme preparations.

[View kit datasheet: www.abcam.com/ab197005](http://www.abcam.com/ab197005)

(use www.abcam.cn/ab197005 for China, or www.abcam.co.jp/ab197005 for Japan)

This product is for research use only and is not intended for diagnostic use.

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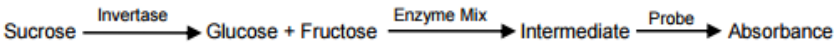
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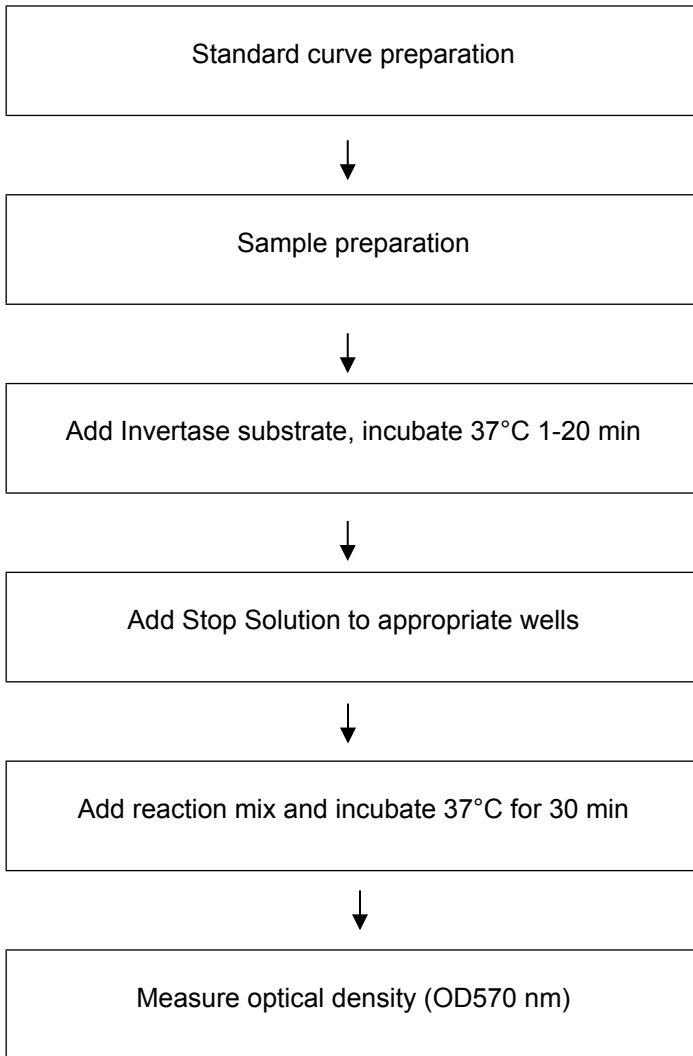
1. BACKGROUND

Invertase Activity Assay Kit (Colorimetric) (ab197005) provides a simple, sensitive and high-throughput adaptable assay that detects relevant concentrations of invertase in biological samples or purified enzyme preparations. The assay is based on the enzymatic detection of glucose, one of TPI's hydrolysis products. The detection limit is 20 μ U.

Invertase (EC 3.2.1.26), also known as sucrase or β -fructofuranosidase, catalyzes the hydrolysis of sucrose (table sugar) by cleaving its glycosidic bond and forming one molecule each of glucose and fructose. Invertase is widely expressed among plants and microorganisms including yeast and bacteria. It is extensively utilized in industrial processes, including food, ethanol production, cosmetics, drugs, paper and bioelectronics electrodes.



2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Invertase Hydrolysis Buffer	25 mL	-20°C	-20°C
Invertase Assay Buffer	25 mL	-20°C	-20°C
Invertase Stop Solution	1 mL	-20°C	-20°C
OxiRed Probe (in DMSO)	200 µL	-20°C	-20°C
Invertase Enzyme Mix	1 vial	-20°C	-20°C
Invertase Substrate	1.2 mL	-20°C	-20°C
Glucose Standard (100 mM)	100 µL	-20°C	-20°C
Positive Control	1 vial	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH₂O)
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD570 nm
- 96 well plate: clear plates for colorimetric assay
- Heat block or water bath
- Sonicator

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Invertase Hydrolysis Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Invertase Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 **OxiRed Probe – in DMSO:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C. Aliquot probe so that you have enough volume to perform the desired number of tests. Store at -20°C protected from light. Once the probe is thawed, use within two months. Keep on ice while in use.

9.4 **Invertase Enzyme Mix:**

Reconstitute in 220 µL Invertase Assay Buffer. Pipette gently to dissolve. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.5 **Invertase Substrate Mix:**

Ready to use as supplied. Aliquot substrate so that you have enough volume to perform the desired number of tests. Store at -20°C. Keep on ice while in use.

9.6 **Glucose Standard:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot standard so that you have enough volume to perform the desired number of tests. Store at -20°C.

9.7 **Positive Control:**

Reconstitute in 140 μ L Invertase Hydrolysis Buffer. Mix well. Aliquot positive control so that you have enough volume to perform the desired number of tests. Avoid repeated freeze/thaw cycles. Store at -20°C . Use within 2 months. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Prepare a 1 mM Glucose standard by diluting 10 μL of the provided 100 mM Glucose standard with 990 μL of ddH₂O.

10.2 Using 1 mM Glucose standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (μL)	Assay Buffer (μL)	Final volume standard in well (μL)	End Conc. Glucose in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 μL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C . When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Bacterial samples:

- 11.1.1 Collect the amount of cells necessary for each assay by centrifugation for 10 minutes at $10,000 \times g$ in a pre-weighted centrifuge tube.
- 11.1.2 Remove supernatant and determine wet weight of the pellet.
- 11.1.3 Resuspend the pellet in Invertase Hydrolysis Buffer using $5 \mu\text{L}$ of Hydrolysis Buffer per mg of sample.
- 11.1.4 Sonicate samples on ice for 1 minute, and keep on ice for 5 minutes.
- 11.1.5 Repeat sonication/ice step 3 times.
- 11.1.6 Centrifuge samples for 20 minutes at 4°C at $10,000 \times g$.
- 11.1.7 Collect the supernatant and keep on ice.

11.2 Biological fluids (Plasma, Serum, Urine or Saliva):

- 11.2.1 Centrifuge samples for 5 minutes at 4°C at $10,000 \times g$.
- 11.2.2 Collect supernatant for use directly in the microplate.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

12.1 Set up Reaction wells:

- Standard wells = 50 μ L standard dilutions.
- Sample wells = 1 – 40 μ L samples (adjust volume to 40 μ L/well with Invertase Hydrolysis Buffer).
- Background Control Sample wells = 1 – 40 μ L samples (adjust volume to 50 μ L/well with Invertase Hydrolysis Buffer). **NOTE:** for samples containing glucose as they can generate background.
- Positive control = 2 – 10 μ L Positive control (adjust volume to 40 μ L/well with Hydrolysis Buffer).

12.2 Substrate Hydrolysis:

- Add 10 μ L Invertase Substrate into each sample and positive control well. (Do not add to background sample control well).
- Mix and incubate for 1 – 20 minutes at 37°C. Record incubation time.

- 12.3 Add 10 μ L Invertase Stop Solution into all wells (standard, samples, positive control and background controls). Mix well.

NOTE: Incubation time depends on the Invertase activity in samples. Longer incubation times may be required for samples having low Invertase activity.

ASSAY PROCEDURE and DETECTION

The table below summarizes the reaction wells set up and substrate hydrolysis step:

Component	Standard well (μL)	Sample well (μL)	Positive control well (μL)	Background well (μL)
Standard	50			
Sample		1 – 40		1 – 40
Positive Control			2 – 10	1 – 10
Hydrolysis Buffer		Up to 40	Up to 40	Up to 40
Invertase Substrate		10	10	
Incubate for 1 – 20 minutes at 37°C [record incubation time]				
Invertase Stop Solution	10	10	10	10

12.4 Reaction Mix:

Prepare 40 μL of Reaction Mix for each reaction:

Component	Reaction Mix (μL)
Invertase Assay Buffer	36
Invertase Enzyme Mix	2
OxiRed Probe	2

Mix enough reagents for the number of assays (samples, standards, positive control and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

$X \mu\text{L component} \times (\text{Number samples} + \text{Standards} + \text{positive control} + \text{background control} + 1)$.

- 12.5 Add 40 μL of Reaction Mix into each samples, standards, positive control and background sample control.
- 12.6 Mix and incubate at 37°C for 30 minutes protected from light.

- 12.7 Measure absorbance at OD=570 nm on a microplate reader.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
 - 13.1 Average the duplicate reading for each standard and sample.
 - 13.2 If the sample background control is significant, then subtract the sample background control from sample reading.
 - 13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
 - 13.4 Plot the corrected absorbance values for each standard as a function of the final concentration invertase.
 - 13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
 - 13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$B = \left(\frac{\text{Corrected absorbance} - (y - \text{intercept})}{\text{Slope}} \right)$$

- 13.7 Invertase activity (in nmol/min/mL or mU/mL) in the test samples is calculated as:

$$\text{Invertase Activity} = \left(\frac{B}{T \times V} \right) * D$$

Where:

B = Amount of Glucose in the sample well (nmol).

V = Sample volume added into the reaction well (mL).

T = Incubation time for substrate hydrolysis (min) (Section 12.2).

D = Sample dilution factor.

Unit definition:

1 Unit Invertase activity = amount of enzyme that generates 1.0 μmol of glucose per minute at pH 4.5 at 37 °C.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

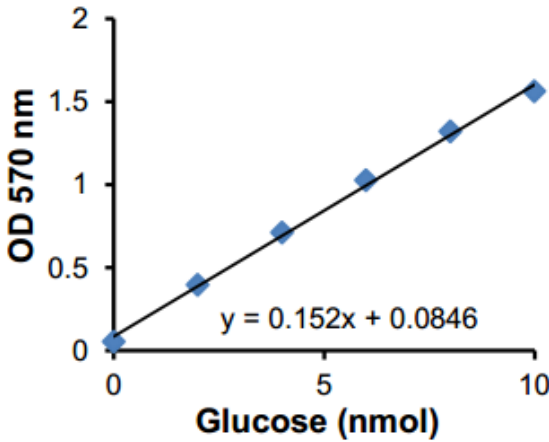


Figure 1. Typical glucose standard calibration curve using colorimetric reading.

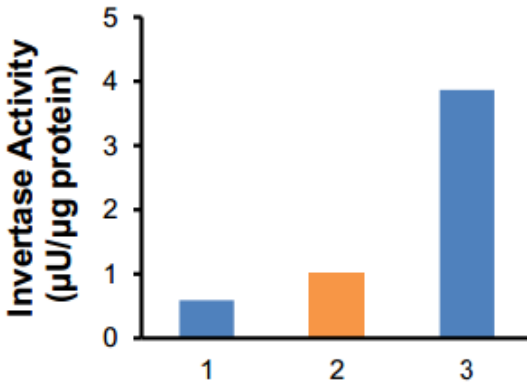


Figure 2: Detection of bacterial Invertase activity in human saliva. Samples collected: 1- Fasting; 2- Low calorie meal and 3: High Sucrose meal. Undiluted

samples (1 and 2 = 25 μ L and 3 = 10 μ L) were incubated for 20 minutes with Invertase substrate.

15. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare buffers, probe, enzyme mix, substrate, stop solution standard and positive control; (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up wells:

Component	Standard well (μL)	Sample well (μL)	Positive control well (μL)	Background well (μL)
Standard	50			
Sample		1 – 40		1 – 40
Positive Control			2 – 10	1 – 10
Hydrolysis Buffer		Up to 40	Up to 40	Up to 40
Invertase Substrate		10	10	
Incubate for 1 – 20 minutes at 37°C [record incubation time]				
Invertase Stop Solution	10	10	10	10

- Prepare Invertase Reaction Mix (Number samples + standards + background control sample + positive control + 1).

Component	Colorimetric Reaction Mix (μL)
Invertase Assay Buffer	36
Invertase Enzyme Mix	2
OxiRed Probe	2

- Add 40 μL of Invertase Reaction Mix to all wells.
- Incubate plate 37°C 30 min protected from light.
- Measure plate at OD570 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

RESOURCES

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 μL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

19. NOTES

RESOURCES

RESOURCES

RESOURCES

UK, EU and ROW

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