



Frequently Asked Questions

Ceralpha: α -Amylase Assay Kit

Q. 1. Can you tell me the analysis method and extraction step for alpha-amylase in potatoes?

A. I believe that if the extraction conditions and assay procedure for wheat alpha-amylase are followed then the assay will work without problems. The pH may need to be adjusted to suit the pH optima of the potato enzyme. Since potatoes contain high levels of polyphenol oxidase, it may be necessary to add some polyvinyl pyrrolidone to bind coloured compounds.

Q. 2. Can the Ceralpha kit distinguish between different forms of alpha-amylase?

A. The Ceralpha Kit does not distinguish between the different forms of alpha amylase, however some idea of relative levels of fungal and bacterial alpha-amylase can be obtained by performing the assay at different pH values. Please refer to the Ceralpha kit booklet on this web site.

Q. 3. In order to use the Ceralpha kit to test for the presence of alpha-amylase, does one have to mill the wheat and if so can I grind the grain in a blender?

A. Cereal grains do need to be milled to allow effective extraction of alpha-amylase (and other constituents). We recommend milling to pass a 0.5 mm screen. You may have some success with a cheap coffee grinder. However, the reproducibility will not be as good (it may be adequate for your requirements).

Q. 4. Can the Ceralpha reagent be used at high temperatures?

A. The Ceralpha method is routinely run at 40°C, but it can be used at temperatures up to 60°C. If it is run at higher temperatures (up to 60°C) then higher activity values will be obtained. This assumes that the enzymes being analysed (alpha-amylase) are stable at these higher temperatures. If you need to measure activity at temperatures above 60°C you can use the Megazyme Amylazyme tablets or Red Starch.

Q. 5. Can Ceralpha Method determine the alpha-amylase activity in a protease preparation?

A. The standard method should be fine. You can best check this by performing a time course hydrolysis of the alpha-amylase. This should be linear if the alpha-glucosidase in the kit reagent is not attacked by the protease.

Q. 6. Can the internal glycosidic linkages only be cleaved by alpha-amylase?

A. Yes, only alpha-amylase can cleave the internal glycosidic bonds.

- Q. 7. After alpha-amylase cleaves the internal glycosidic linkage, can beta-amylase or glucoamylase accelerate the hydrolysis reaction of the released p-nitrophenyl maltosaccharide?
- A. When alpha-amylase cleaves the glycosidic linkage in the blocked substrate, any other enzymes in the extract do not accelerate the hydrolysis to give free p-nitrophenol. The reason for this is that the level of thermostable alpha-glucosidase in the substrate mixture is saturating.
- Q. 8. Can Rice alpha-amylase be analyzed by Ceralpha kit?
- A. Rice alpha-amylase can be assayed with Ceralpha kit. Alpha-glucosidase in the rice extract will not interfere.
- Q. 9. Why is extraction conducted at room temperature or 40°C, and not at 4°C?
- A. We have found that we get effective extraction from a range of cereal flours at room temperature (approx. 22°C) and that the enzyme was very stable at this temperature for several hours. For wheat flour, the optimal temperature for extraction is 40°C (refer to the Ceralpha Booklet).
- Q. 10. Can the Ceralpha method be used to determine alpha-amylase from oat flour extracts?
- A. The Ceralpha method can be used for oats. The only likely problem is the high viscosity of the extract. If the extract looks very viscous, double the ratio of extraction buffer to flour. Allow for this in the calculations. If the absorbances are low then increase the incubation time to say 30 minutes. Then allow for this increased time of incubation in the calculations.
- Q. 11. Is there a method to increase the sensitivity of assay regarding the measurement of alpha-amylase in fermentation broth?
- A. I suggest that the fermentation broth is simply adjusted to 5.0 with acid or base and this can be used directly in the assay. The sensitivity can then be increased by increasing incubation time up to 4 hours. Appropriate adjustments then need to be made to the calculations.
- Q. 12. Can the reaction blank be used to zero the spectrophotometer directly?
- A. You can zero the spectrophotometer directly with the blank. However, it is wise to know how high the blank is to ensure that the substrate is OK; i.e. the blank is usually 0.05-0.06. If it is above 0.1, it has probably been contaminated with some alpha-amylase enzyme.
- Q. 13. When the absorbance is between 1.0 – 1.5, can the solution be diluted directly with an additional 3.0 ml of distilled water to cut the absorbance in half before reading it?
- A. If the absorbance value is above 1.2 then the assay will be limited by the amount of available substrate. Thus, you cannot simply dilute the colour.

Q. 14. I have immobilized alpha-amylase onto glass bead and now I want to test the activity, can the Ceralpha kit test for this?

A. To measure alpha-amylase on glass beads I would recommend the use of the Ceralpha method. The kit is complete and extremely easy to use. Perhaps to assay, you may freeze dry a small sample of the beads and then weigh an appropriate amount (say 5 mg) into a test tube. Add 0.2 ml of buffer pH 5-6 and 0.2ml Ceralpha reagent. Incubate at 40°C for 10 min.

Q. 15. Is it possible to measure acid-stable alpha-amylase by Ceralpha Kit?

A. The Ceralpha method should be fine for acid stable alpha-amylase. However, assays should be performed in the pH range of 5.2 to 7.5.

Q. 16. Can you tell me the application of the Amylazyme Tabs and Ceralpha Kit to residual enzymes in finished bakery products, particularly in sweet goods?

A. For measurement of residual enzymes in bakery products, you should use the Amylazyme method rather than the Ceralpha method. With the standard assay formats available, the sensitivity of the Amylazyme method is 10-20 times greater than can be achieved with the Ceralpha method. You will need this greater sensitivity to measure the extremely low levels of activity present.

Q. 17. What would be an expected level of Ceralpha units in germinated or malted barley?

A. The levels of alpha-amylase in malted barley is about 150 Ceralpha units per gram, which would be the expected level in germinated barley grain.

Q. 18. We want to check for extremely low levels of alpha-amylase in dry powdered ingredients containing starch, sugar, and non-fat dry milk. Our main challenge with your Ceralpha Assay Kit would be clarifying samples so we can read them on a spectrophotometer. Any suggestions?

A: I am aware of the problem of measurement of trace levels of alpha-amylase in starch based sources. The Ceralpha method may not be sensitive enough for these assays. I suggest that you purchase the Amylazyme method (run Format B). You can get 10 x greater sensitivity with this assay.

Q. 19. We would like to obtain an assay capable of quantifying trace amounts of alpha-amylase activity in the pullulanase preparation. Please let me know if Megazyme has any suitable assay available.

A: I think the Ceralpha Reagent would probably be best for this application.

Q. 20. I am interested in the Amylazyme and Ceralpha available from Megazyme. Are they applicable not only for cereal and microbial alpha-amylase but also porcine pancreatic alpha-amylase?

A: The Ceralpha method is excellent for all alpha-amylases and will work fine for porcine pancreas alpha-amylase at pH 6.9.

Q. 21. We would like to know where the coefficient of 18.1 (E_{mM} of *p*-nitrophenol) comes from? What does it mean?

A: This is the extinction coefficient, i.e. absorbance of a 1 mM solution of *p*-nitrophenol in a 1 cm light path at 400 nm.

Q. 22. I wish to measure the enzyme activity of alpha-amylase in bread after cooking. Will your Ceralpha-Amylase Kit work on cooked breads? If so, do I need to alter the procedure?

A: The level of alpha-amylase in wheat flour is quite low, so in bread baked from this flour it is likely to be much lower. The Ceralpha test is very sensitive, but you can increase sensitivity by increasing incubation time up to several hours (6 hours). This should allow measurement of the enzyme if there is any there.

Q. 23. Any recommendations on using your Ceralpha alpha-amylase test at a low pH (pH 3-4)?

A. Cereal alpha-amylase assay reagent cannot be used at pH 3-4. However, you can perform a similar assay using blocked *p*-nitrophenol maltoheptaoside (BPNPG7). Basically dissolve the BPNPG7 vial contents in 10ml with water. Incubate 0.2 ml of your enzyme (buffered) with 0.2 ml of BPNPG7 for 10 min at a set temperature. Terminate the reaction by heating in a boiling water bath for 2 min. Then add 0.2 ml of alpha-glucosidase (E-TSAGL; at 10 U/ml in 0.2M tris buffer, pH 7.0) and incubate for 10 min. Add 3 ml stopping reagent (Trizma base pH 8.5) and read colour at 400nm