Frequently Asked Questions

Total Starch Assay Kit

Q. 1: Why does the quadruplicate glucose control in your Total Starch Assay kit have to be incubated?

A: We feel more comfortable with quadruplicate glucose controls. If the control is incorrect, or questionable, then all the results are in doubt.

Q. 2: With regard to your Total Starch Assay Kit, can you please tell me why duplicate samples have to be measured?

A: Duplicate samples do not have to be measured. We just suggest this for labs starting up.

Q. 3: We are currently using your kits for Total Starch, Starch Damage and Sucrose/Glucose/Lactose Assay procedures. I have a query regarding the enzyme vials – how accurate are the volumes contained? Can they be diluted entirely, to avoid wasting the contents while transferring?

A: When we dispense the enzymes we usually include an extra 5% in each vial, so yes, you can dilute the whole vial. When you do this please divide into aliquots and store them frozen.

Q. 4: I would greatly appreciate if you could let me know whether you have any kit or procedures for the determination of extractable starch in corn. This is of particular concern in the corn wet milling. I presume that extractable starch in corn is not the same as total starch?

A: Our Total Starch Assay Kit could measure starch left in a residue, or starch extracted. No method could measure potential extractable starch, as this will depend on numerous factors, including processing equipment, conditions etc.

Q. 5: Could your Total Starch Assay Kit (K-TSTA) be used with success to measure total starch in plant tissues (samples of roots and shoots of maple trees)?

A: Yes, the Total Starch Kit can be used to measure starch in roots and shoots etc.

Q. 6: We are interested in some analytical procedure for determination of total starch residues in brewing wort and final beer. Since Megazyme's procedure – AA/AMG'97, only refers to solid (and not solubilised) starch, which would be your recommendations regarding this request?

A: You can use the standard procedure. I would recommend that you treat 2ml of beer or wort with 8 ml of ethanol, stir and centrifuge (3,000 rpm). Wash the pellet with 10 ml of 80% ethanol. Then dissolve/suspend the pellet in 2 ml of sodium acetate buffer (pH 4.5, 0.1 M) and cook at 100^{o}C for 10 min. Then add 0.1 ml AMG from the total starch kit and proceed according to the method. You will have to determine the degree of dilution for yourself. Treat 0.1 ml with GOPOD etc.

Q. 7: Can I use another buffer instead of the MOPS with your Total Starch Assay Kit? If so, which would be suitable and easily prepared from commonly available laboratory reagents?

A: You can use phosphate buffer at the same concentration.

Q. 8: I wish to measure Total Starch in several products. These products contain 10-20% starch + maltodextrins at similar levels. Is it possible to remove the maltodextrins from the sample? Will ethanol work?

A: Most of the maltodextrins can be removed with 50% ethanol washing. If the starch is not gelatinised, it can be washed with cold water. This will remove all of the soluble maltodextrins, but the starch will spin down. If the starch has been gelatinised, then the best material which can be used for washing is 50% ethanol.

Q. 9. Is the accuracy of the Total Starch test affected by the presence of other inorganic chemicals and ground calcium carbonate in pulp?

A. I think that calcium carbonate etc. will not cause any problems. However, this of course depends on the amount present and if it changes the pH of the incubation mixture.

Q. 10. Does the Megazyme Total Starch method work well on all the new chemically modified starches that are now appearing e.g. highly crosslinked, dextrinised and highly propylene oxide substituted?

A. The method will work for some chemically modified starches (e.g. crosslinked) however, if the degree of chemical modification is high, there will be an underestimation as the modification will interfere with complete hydrolysis to glucose and subsequent measurement.

Q. 11. Is it necessary to pre-wash ground cereal samples prior to analysis for Total Starch?

A. You only need to wash samples which you feel may contain glucose and/or maltodextrins e.g. breakfast cereals. There is little glucose in ground cereals, so it is not necessary to pre-wash these materials.

Q. 12. What is the stability of the enzymes from the Total Starch Kit?

A. The enzymes from this kit are stable at room temperature for at least 6 months. At 4^{o}C, they are stable for several years.

Q. 13. Can the Total Starch Kit determine the degree of gelatinisation? Sample : Corn Flour

A. The Starch Damage Kit may be best for this if the starch is gelatinised and dried before analysis the correct results for gelatinisation will not be obtained.

Q. 14. Are there any limits to the sensitivity of the Total Starch kit?

A. The Total Starch Kit can accurately measure starch levels as low as 1% w/w?

Q. 15. What is the sensitivity and how much is the absorbance of glucose standard (100 micrograms)?

A. The absorbance for 100 micrograms of glucose (in 3 ml of GOPOD Reagent) is about 0.97.

Q. 16. Is it possible to raise sensitivity by modifying dilution of GOPOD reagent?

A. Yes, you can reduce the volume of GOPOD to 1ml and use micro cuvettes. This will increase sensitivity by ~ 3-fold.

Q. 17. Does DMSO solubilise resistant starch i.e. crystallised amylose and amylopectin?

A. DMSO does solubilise resistant starch (crystallise amylose and amylopectin). The only starch material we have had problems in dissolving in DMSO is potato amylose.

Q. 18. When analysing samples containing sugars, an 80% v/v solution of ethanol is used to solubilise and remove the sugars. About how large are the smallest dextrins that are left in the starch (not solubilised) in this treatment?

A. I believe that for starch fragments, oligosaccharides of a DP up to 10 would be soluble in 80% alcohol. The degree of solubility of other oligosaccharides would depend on the sugar type and linkage type.

Q. 19. What is the sensitivity of the Total Starch Method for measurement in liquids containing low levels of starch?

A. The Starch Kit can be used for liquids containing as little as 200 micrograms per ml with some adjustments of conditions, as below:

Mix 0.5ml of sample with 0.5 ml of 100 mM sodium acetate buffer (pH 4.5). Incubate at 40^{o}C and add 0.1ml of Amyloglucosidase and incubate for 30 minutes. Add GOPOD reagent as usual. You will need to run an AMG blank as this enzyme preparation contains a very small amount of glucose.

Q. 20. AMYLOGLUCOSIDASE. The activity is stated as being 3260 U/ml (Soluble Starch). How was this determined?

A. The AMG activity was determined with soluble starch as substrate (10mg/ml) in 0.1 M sodium acetate buffer at pH 4.5 and 40^{o}C. One Unit is the amount of enzyme required to hydrolyse one micromole of maltose per minute (i.e. to release 2 micromoles og glucose). Glucose release is measured with Glucose Determination Reagent.

Q. 21. I wish to know if it is possible to perform the assay under acidic conditions? I also need to alter the pH of the MOPS/amylase mixture to pH 3 or 4. Is it known if the amylase supplied with the Total Starch Assay Kit has activity at such a low pH?

A. I can assure you that the Total Starch Kit will not work if incubations with the thermostable alpha-amylase are performed at pH 3 or 4. This enzyme is inactivated at pH values below 5.0. You may wish to look at a method using just amyloglucosidase which is quite active down to pH 4.0. Check the old AOAC procedure for starch.

Q. 22. Does the Regular Maize Starch need to be analysed with pre-treating by DMSO? How do you store this Enclosed Control?

A. The Regular Maize Starch does not require DMSO pre-treatment. The value should be about 84% with a moisture content of about 12%, the final dry weight value is about 96-97%. Store the sample at room temperature, dry.

Q. 23. Does your Kit with DMSO solubilise starch that has been vitrified due to malting/kilning?

A. Yes. I believe that the DMSO step will solubilise vitrified starch in malt. Make sure that the malt is milled to pass a 0.5 mm screen. You could vary the time of cooking with DMSO to check solubilisation (i.e. 5 minutes, 10 minutes, or even up to 1 hour)

Q. 24. Is it possible to differentiate between gelatinised and ungelatinised starch in finished products such as dog food using the Total Starch Kit?

A. I think that there is a better chance of success using the Megazyme Starch Damage kit.

Q. 25. Is it possible to use the Total Starch Kit to measure starch levels in plasterboard and related products?

A. There should be no problem in measuring the starch in plasterboard. I suggest that you grind about 100 g in a kitchen blender and then fine mill to pass 0.5mm screen. Run a standard assay, but adjust volume to 10 ml after alpha-amylase treatment. Keep a close check on the pH. Plasterboard may push the pH value up (pH up to about 8 should be fine). You may be advised to run a DMSO format concurrently just to be sure. When you treat with amyloglucosidase, I would advise that you take 0.2 and 0.4 ml aliquots of digest (to get the colour up), also, be careful about checking the pH.

Q. 26. Can the Total Starch Kit be used for samples containing 20% fat or higher?

A. A 20% fat content could cause a problem for the method. I suggest that the sample be defatted before analysis for starch.