



Frequently Asked Questions

Fructan Assay Kit

- Q. 1. When measuring fructan in starch containing samples i.e. maize, is there a possibility of incomplete starch hydrolysis in step B?**
- A. From our experience, the level of beta-amylase/pullulanase etc. added is more than adequate to completely hydrolyze any starch likely to be present. However, if there is a suspicion of incomplete starch hydrolysis, you can check this by running the hydrolysis with the pullulanase/beta-amylase etc. over a range of incubation times and comparing the results.
- Q. 2. Could you tell me whether this method simply gives a gross measure of the fructan content, or whether it is possible to specifically determine the inulin content of the material?**
- A. The method measures inulin and cereal fructans.
- Q. 3. Can the fructan method be used to measure inulin in starch-containing products i.e. Cornflakes?**
- A. Yes.
- Q. 4. What typical absorbance values are obtained for fructan control and fructose control?**
- A.

1. Fructan Control Flour (29.6%)	0.496	0.498
2. Fructose Control	1.133	1.135
- Q. 5. What is the precision of the method for measurement of fructans?**
- A. Our reproducibility is usually +/- 5 to 7% of the fructan value.
- Q. 6. Do you have statistics for the variability of your diagnostic test kits? I would like to get an idea what typical %RSD values would be for your oligofructan test kit.**
- A. With all our kits we can obtain approx. 5% c.v. in interlaboratory studies. In-house these values are closer to 3%.
- Q. 7. Can I detect the levan content of some plant materials by use of your fructan kit?**
- A. The fructanase in the kit will completely hydrolyse the fructans in plants. These are either inulin "type" or "branched" type (as in oat or wheat stems). I do not know if the enzymes would act on pure levan.

- Q. 8. What actually is the factor value (F)? Is it always 54.5 or does it change depending on the fructose content?
- A. The value of 54.5 is the actual amount of fructose standard used. This amount of fructose will give a certain colour in the PAHBAH assay.
Thus; $F = \text{factor} = [54.5 \text{ (micrograms of fructose)}] \text{ divided by the absorbance value obtained for 54.5 micrograms of fructose in the PAHBAH method}$
- Q. 9. In step 5d the procedure states that the pH will be close to 4.5. When I check this, I find it to be consistently in the range of 8-9.
(a) Does this seem correct?
(b) Would it affect the outcome of the test?
- A. The pH should be 4.5. I suggest that you check the concentration of the acetic acid that you are using. Incorrect pH at this step will result in lower than expected values.
- Q. 10. Sucrase" which is absolutely specific for the cleavage of sucrose without touching any of the other potentially present oligo/polysaccharides (mixed linked glucan, soluble starch, fructan of any size and structure. Did I understand this right? And is there a possibility to obtain that enzyme separately?
- A. Yes, the sucrase does hydrolyse sucrose only and has no action on the other carbohydrates that you mention. The "sucrase" enzyme is an alpha-glucosidase which we sell as Alpha-Glucosidase (Maltase).
- Q. 11. Is it possible to buy Fructanase separate from the kit?
- A. Yes. The best enzyme to use for analytical purposes is exo-Inulinase (E-EXOI).
- Q. 12. We need to analyse sucrose in extracts of chicory roots and the sucrase used in your fructan assay procedure may solve the problem? Do you think that it would be possible to use sucrase instead of invertase in the sucrose assay?
- A. Yes, the sugar enzyme can be used to measure sucrose. The enzyme is an alpha-glucosidase, so it will also hydrolyse maltose if this is present.
- Q. Does the fructanase enzyme hydrolyse Neosugar?
- A. Yes
- Q. 13. Is it possible to do the weighing and hot extraction the day before the actual assay.
- A. Extract solutions are best made and analysed the same day (not stored overnight). But if they are stored overnight in a refrigerator, heat them to 80°C, and cool before analysing (to ensure all fructan has re-dissolved)

- Q. 14. Regarding your Fructan Assay Procedure - your instructions Step 3, Page 9 state that "if the sample is cooled, it must be reheated to 80°C to ensure that the fructans redissolve". If the samples are frozen to -20°C and then thawed quickly and reheated to 80°C, will the fructans re-dissolve completely?
- A. Fructans are very soluble and will redissolve very readily. They may precipitate on freezing (at least the polymeric portion), but they will easily re-dissolve at 80°C.