BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Fructose Assay kit (no glucose; Cat #: A-135)

COMPONENTS: Fructose Assay Solution: 5 ml (for 100 wells); store at -80°C **(shield solution from light during use)**
20 mM Fructose: 0.5 ml, store at -20°C or -80°C
PEG Solution: 3 ml; store at room temperature **(agitate vial to redissolve contents upon arrival)**

PRODUCT DESCRIPTION: The Fructose Assay Kit is based on conversion of F-6-P to G-6-P followed by coupled G6PD and PGI reactions. The kit is used to measure sample fructose concentration (linear range 10 – 500 µM) in the absence of glucose. Please refer to A-112 for samples containing glucose. PEG Solution is used to remove proteins, which can interfere with the assay. The kit is stable for several years if stored and handled properly.

PROTOCOLS

Sample deproteination: Samples containing proteins are first deproteinized by PEG precipitation. Mix 25 µl of each sample with 25 µl PEG Solution in a 1.5-ml microtube (PEG solution should be pipetted slowly using a cut tip). Shake tube vigorously followed by vortexing to ensure thorough mixing. Keep tube on ice for 3 min. Centrifuge solution in a microfuge at ~14,000 rpm for 3 min. Harvest supernatant for fructose assay. Store deproteinized samples at -20°C.

Fructose standard: Dilute the 20 mM fructose standard 100-fold with dH_2O to 200 µM, e.g., 495 µl dH_2O + 5 µl 20 mM glucose/fructose. Perform 1:1 serial dilution to generate 100, 50, and 25 µM standards. Store diluted standards at -20°C.

Reagent thawing: Quickly thaw Fructose Assay Solution and keep solution on ice and shielded from light. *Do not over thaw solution.* Freeze solution immediately after use.

ASSAY

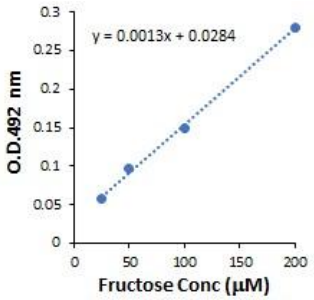
1. Add 20 µl of each fructose standard and sample to a plain (uncoated) 96-well microplate.

2. Add 50 µl Fructose Assay Solution to each fructose standard well and sample well. Mix contents by gentle agitation for 10 sec. Cover plate and incubate at 37°C for 30 min. *Do not use CO_2 incubator.*

3. Eliminate air bubbles present in wells prior to measurement if necessary. Measure absorbance at 492 nm using a plate reader.

4. Plot fructose standards (x axis) vs. $O.D._{492\ nm}$ (y axis). Generate a trend line equation on chart for fructose standard (see graph below).

5. Calculate sample fructose concentration using the derived equation. x = fructose concentration in µM; y = $O.D._{492\ nm}$. A new equation must be established for each assay. Multiply sample fructose concentration by the applicable dilution factor.



ADDITIONAL INFORMATION:

- Fructose Assay Solution contains the organic solvent dimethyl sulfoxide (DMSO) and iodonitrotetrazolium chloride. Please contact us or visit the product webpage for material SDS information.
- Agitate the PEG vial in warm water to re-dissolve contents upon arrival.