# *BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO*

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## Fructose Assay kit (with glucose; Cat #: A-112)

**COMPONENTS**: FG Assay Solution: 5 ml (for 100 wells); store at -80°C **(shield solution from light during use)**
Glucose Assay Solution: 5 ml (for 100 wells); store at -80°C **(shield solution from light during use)**
20 mM Glucose: 0.5 ml, store at -20°C or -80°C
20 mM Fructose: 0.5 ml, store at -20°C or -80°C
GOX Solution: 0.25 ml, store at -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 fructose and glucose present in biological fluids and culture medium (linear range 10 – 500 μM). The kit is stable for several years if stored and handled properly.

## PROTOCOLS

**Reagent thawing:** Quickly thaw FG Assay Solution and Glucose Assay Solution. Keep solutions on ice and shielded from light. *Do not over thaw solutions.* Freeze solutions immediately after use.

**GOX treatment:** Samples containing glucose, such as serum/plasma and culture medium, are pretreated with GOX prior to fructose assay. Pipette 50 μl of each sample to a microtube and mix with 2.5 μl GOX Solution. Vortex tube for 10 sec (to promote oxygenation). Incubate at 37°C for 90 min with intermittent vortexing.

**Sample deproteination:** GOX-treated samples can be deproteinized by PEG precipitation if necessary. Mix 50 μl of each sample with 50 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 and store samples at -20°C.

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

## ASSAY

1. Add 20 μl of each diluted glucose standard and fructose standard to a plain (uncoated) 96-well microplate. Add each sample to duplicate wells of the plate.

2. Add 50 μl FG Assay Solution to each fructose standard well and one set of sample wells. The setup serves to measure sample fructose+glucose concentrations.

3. Then add 50 μl Glucose Assay Solution to each glucose standard well and the other set of sample wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate at 37°C for 30 min. *Do not use $CO_2$ incubator.*

4. Measure absorbance at 492 nm using a plate reader. Plot glucose and fructose standard concentrations (x axis) vs. $O.D._{492\ nm}$ (y axis). Generate a trend line equation on chart for glucose standard and fructose standard.

5. Calculate sample glucose concentration using the derived glucose equation (x = glucose concentration in μM; y = $O.D._{492\ nm}$). Calculate sample fructose+glucose concentration using the derived fructose equation. Multiply concentration by the applicable dilution factor.

6. Sample fructose concentration = $\mathbf{[fructose+glucose]_{FG\ Assay\ Solution\ well} - [glucose]_{Glucose\ Assay\ Solution\ well}}$

**ADDITIONAL INFORMATION:**

- Both FG Assay Solution and Glucose Assay Solution contain 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.