

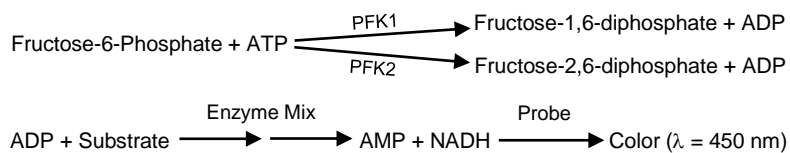


Phosphofructokinase (PFK) Activity Colorimetric Assay Kit rev. 11/15

(Catalog # K776-100; 100 assays; Store at -20°C)

I. Introduction:

Phosphofructokinase (PFK) is a key glycolytic enzyme and plays a major regulatory role during glycolysis. This enzyme is present in bacteria, plants and animals. There are 2 types of PFKs - PFK1 and PFK2. In the presence of ATP, PFK1 & PFK2 catalyzes the conversion of fructose-6-phosphate to fructose-1,6-diphosphate and fructose-2,6-diphosphate respectively and ADP. PFK has 3 major isoforms in mammals: PFK-M (muscle), PFK-L (liver) and PFK-P (platelet). In humans, PFK deficiency causes glycogen storage disease, also called Tarui's disease, which is characterized by exercise-induced muscle weakness and cramps. On the other hand, increased PFK activity contributes to cancer cell proliferation and tumorigenicity. Early detection of abnormal phosphofructokinase activity is crucial for diagnosis, prediction and therapeutic strategy. In BioVision's Phosphofructokinase Activity Assay kit, PFK converts fructose-6-phosphate and ATP to fructose-diphosphate and ADP. The ADP in the presence of substrate and enzyme mix is converted to AMP and NADH, which reduces a colorless probe to a colored product with strong absorbance at 450 nm. PFK activity assay is simple, robust, and rapid and can detect phosphofructokinase activity less than 1 mU.



II. Application:

- Measurement of phosphofructokinase activity in various tissues/cells.
- Analysis of glucose metabolism and cell signaling in various cell types.
- Screening anti-cancer drugs.

III. Sample Type:

- Animal tissues: Liver, Brain, Heart, Muscles etc.
- Cell culture: Adherent or suspension cells.

IV. Kit Contents:

Components	K776-100	Cap Code	Part Number
PFK Assay Buffer	27 ml	WM	K776-100-1
PFK Substrate (lyophilized)	1 vial	Blue	K776-100-2
ATP (lyophilized)	1 vial	Orange	K776-100-3
PFK Enzyme Mix (Lyophilized)	1 vial	Green	K776-100-4
PFK Developer (Lyophilized)	1 vial	Red	K776-100-5
NADH Standard (Lyophilized)	1 vial	Yellow	K776-100-6
Positive Control (Lyophilized)	1 vial	Purple	K776-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at -20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **PFK Substrate:** Reconstitute with 220 µl Assay Buffer. Store at -20°C. Use within two months. Keep on ice while in use.
- **ATP:** Reconstitute with 220 µl dH₂O. Store at -20°C. Use within two months. Keep on ice while in use.
- **PFK Enzyme Mix:** Reconstitute with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.
- **PFK Developer:** Reconstitute with 220 µl dH₂O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 40 µl Assay Buffer to generate 10 mM NADH Standard stock solution. Store at -20°C. Use within two months. Keep on ice while in use.
- **Positive Control:** Reconstitute with 100 µl Assay Buffer and mix thoroughly. Aliquot and store at -20°C.

VIII. Phosphofructokinase Activity Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (20 mg) or cells (2 x 10⁶) with 200 µl ice cold Assay Buffer on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (100 µg) per well. Adjust final volume to 50 µl with Assay Buffer. Prepare a parallel sample well as the background control to avoid interference from ADP and NADH in the sample.

Note: a) Small molecules in some samples such as liver may interfere with PFK Activity Assay. ADP, NADH and other interfering small molecules can be removed by 10 KD spin column (BioVision Cat# 1997-25). Specifically, pre-wet the spin column with dH₂O, spin down

