



**KPG Ferric Reducing Ability of Plasma (FRAP) Assay Kit (For research use only)**

**Catalog Nos. KPG-FRAP Lot Nos. KPG-FRAP1**

**Component of KPG NO Assay Kit:**

- Solution A (CN. KPG-Sa) , 1 Vial 3 mL
- Solution B (CN. KPG-Sb), 1 Vial 3 mL
- Solution C (CN. KPG-Sc), 1 Vial 3 mL
- Standard (CN. KPG-StdFRAP), 1 Vial 2 mL

**Other materials needed:** Samplers, Centrifuge, ELISA plate and ELISA reader covering 593 nm.

**Preparation of the solution:** Solution A, B and C needs to be mixed immediately before experiments in 10/1/1 ratio to make working solution. For example, if you need to test 4 samples prepare 300  $\mu$ L working solution, so mix 250  $\mu$ L solution A with 25  $\mu$ L solution B and 25  $\mu$ L solution C. The mix is stable for 30 minutes only and needs to be prepared immediately before experiments.

**Preparation of standards**

Add 100 mL deionized distilled water (DDW) to the standard bottle and mix well to make 10 mM solution. Add 2 mL from the standard with 10 mM  $\text{Fe}^{2+}$  to 8 mL DDW to make a 2 mM concentration and then make serial dilutions using the following table to make standard 1 to 6:

Std	Concentrations ( $\text{Fe}^{2+}$ )	$\text{H}_2\text{O}$	volume
1	1 mM (1000 $\mu$ M)	500 $\mu$ L	500 $\mu$ L
2	0.7 mM (700 $\mu$ M)	650 $\mu$ L	350 $\mu$ L
3	0.5 mM (500 $\mu$ M)	750 $\mu$ L	250 $\mu$ L
4	0.3 mM (300 $\mu$ M)	850 $\mu$ L	150 $\mu$ L
5	0.2 mM (200 $\mu$ M)	900 $\mu$ L	100 $\mu$ L
6	0.1 mM (100 $\mu$ M)	950 $\mu$ L	50 $\mu$ L

**Procedure:**

1. Before starting the procedure keep the kit components in room temperature for 20 minutes.
2. Add 5  $\mu$ L sample, standards and DDW (as blank) to the clean ELISA plates, then add 70  $\mu$ L working solution and mixed for 30 seconds.
3. Incubate the plate at 37  $^{\circ}\text{C}$  for 5 minutes and evaluate its OD at 593 nm.

**Safety directions:** The solutions are tissue hazard materials, hence, in the cases of contact with skin, eyes and ETC, wash with water and refer to hospital for additional medications.



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