JASEM

www.jasem.com.tr

Glutathione in Whole Blood LC-MS/MS Analysis Kit

Glutathione is a natural tripeptide (γ-L-glutamyl-L-cysteinylglycine) which participates in several physiological processes such as DNA synthesis, protein synthesis, cell membrane stabilization, amino acid transportation and xenobiotic detoxification. Glutathione exists in reduced form (GSH) and in oxidized form as disulfide (GSSG). The GSH/GSSG ratio is often used as a marker of the cellular redox state. GSH is a critical indicator protecting organisms against diseases and toxicities. Thus, measurements of GSH concentrations in biological samples are of importance, owing to its potential for disease diagnosis and providing valuable information for the understanding of GSH homeostasis.

Highlights of the Analysis Kit

- For the determination of total glutathionine level, a simple reduction step is apllied prior to protein precipitation. No need of chemical derivatisation
- Total run time is 10.5 min.
- Safeguarded by its own stable labelled isotopic internal standard
- Small volume of patient's sample is required
- Long life span of HPLC column

Parameters			
Total Glutathione (GSH+GSSG)	Glutathione (GSH)	Glutathionedisulfide (GSSG)	GSH/GSSG
Sample Type			
Whole Blood			

Sample Preparation for Total Glutathione

1	Pipette 20 µL of whole blood or quality control sample into a glass centrifuge tube then, add 40 µL of Reagent-1 and vortex for 15 sec. Next, incubate in a water bath at 60 oC for 15 min.
2	Let the tube cool down to the room temp and dilute by adding 1940 µL of Reagent-2, vortex 3 sec.
3	Transfer 50 µL of diluted sample into a new tube. Then, add 25 µL of internal standard and 225 µL of Reagent-3 respectively, vortex 3 sec. Subsequently, centrifuge at 4000 rpm for 3 min.
4	Decant the supernatant into HPLC vial prior to injection

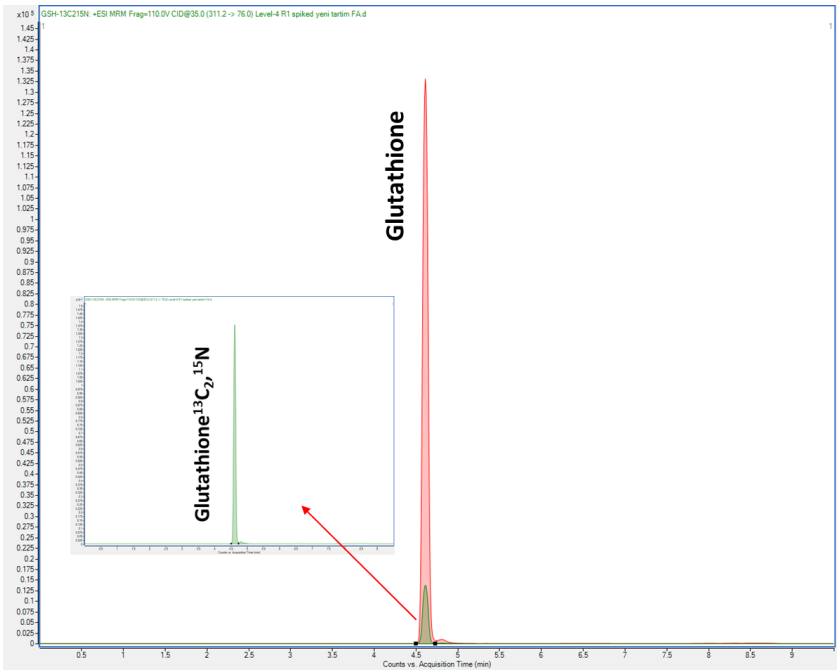
Sample Preparation for Free Glutathione

1	Pipette 20 µL of whole blood or quality control sample into a glass centrifuge tube and add 1980 µL of Reagent-2, vortex 3 sec.
2	Transfer 50 µL of diluted sample into a new tube. Then, add 25 µL of internal standard and 225 µL of Reagent-3 respectively, vortex 3 sec. Subsequently, centrifuge at 4000 rpm for 3 min.
3	Decant the supernatant into HPLC vial prior to injection

Calculation the amount of oxidised glutathione (GSSG)

$$GSSG = \frac{(\text{Total GSH-Free GSH})}{2}$$

Example Chromatogram



Total ion chromatogram

Method Performance

All results were obtained using Agilent 6470 TQ system

Analytes	LOQ (µmol/L)	Linearity (µmol/L)	Recovery		Repeatability			
					intra-day		inter-day	
			LLQC* (%)	HLQC** (%)	LLQC (%CV)	HLQC (%CV)	LLQC (%CV)	HLQC (%CV)
Total Glutathione	0.14	0.2 – 30.0	101	96	0.14	0.04	7.60	4.98
Free Glutathione	0.19	0.2 – 30.0	100	97	0.19	0.06	7.47	5.53
GSSG	0.12	0.2 – 30.0	105	86	0.12	0.03	7.98	7.36

* LLQC: Low-level quality control
** HLQC: High-level quality control

Altium International Laboratuvar Cihazları A.Ş.
Barbaros Mah. Temmuz Sk. No:6 Altium Plaza Ataşehir, İstanbul
T: +90 216 571 02 00 F: +90 216 571 02 02