

Comparison of the BD™ P800 and BD™ P700 Blood Collection Systems, BD Vacutainer® K₂EDTA tube, and other Protease Inhibitors for the Preservation of Active GLP-1

OVERVIEW

Blood from 4 individual subjects was drawn into P700, P800, and K₂EDTA BD evacuated blood collection tubes. Immediately after collection, Linco™ DPP-IV inhibitor or aprotinin was added to designated K₂EDTA tubes from each subject. The tubes were immediately centrifuged and the plasma pooled from each tube/inhibitor type collected.

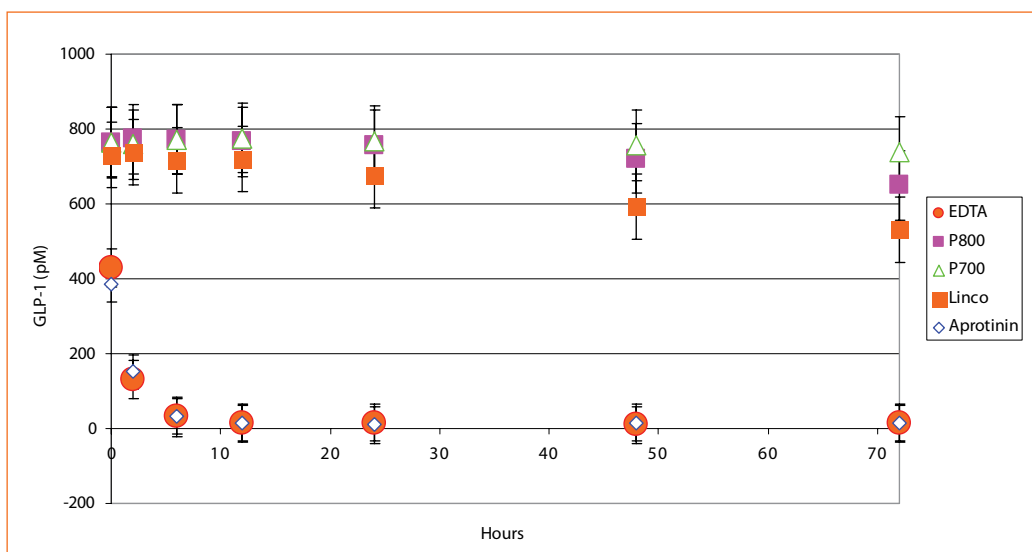
An equivalent volume of plasma from each tube/inhibitor type (P700, P800, K₂EDTA, K₂EDTA+Linco™ DPP-IV inhibitor, and K₂EDTA+aprotinin) was spiked within 1 hour of collection with 800 pM of Active GLP-1 (7-36 amide). Each set of plasma pools prepared from each subject was maintained at 25±3°C. Plasma aliquots were taken at 0, 2, 4, 6, 12, 24, 48 and 72 hours and stored frozen below -60°C until assayed for Active GLP-1 by ELISA.

RESULTS

The mean subject Active GLP-1 assay results at each time point are found in **Figure 1**. The data (**Table 1**) demonstrate the following:

- Comparable GLP-1 stability was observed for P700 and P800 tubes at 0, 2, 6, 12, 24 and 48 hours of 25±3°C storage.
- Comparable GLP-1 stability was observed for P700, P800 and K₂EDTA+Linco™ DPP-IV inhibitor tubes at 0, 2, 6, 12 and 24 hours of 25±3°C storage.
- Immediate and rapid degradation of active GLP-1 was observed for K₂EDTA and K₂EDTA+aprotinin tubes at all time intervals (hours) post spiking with active GLP-1 concentrations and returning to baseline concentrations within 12 hours of 25±3°C storage.
- Baseline results were consistently higher in P700 and P800 compared with Linco, aprotinin and K₂EDTA additives.

Figure 1.
Active GLP-1 (ELISA)



*Note: Active GLP-1 was spiked into plasma after addition of the Linco inhibitor.



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Table 1. Active GLP-1 ELISA Data PAS-09-TUB020

Stability Assessment of Plasma GLP-1 Concentration in the Presence of Various GLP1 Inhibitors											
Subject 1						Subject 2					
Time (Hr)	EDTA	P800	P700	Linco	Aprotinin	Time (Hr)	EDTA	P800	P700	Linco	Aprotinin
Baseline	14	19	19	16	14	Baseline	15	18	18	15	18
t0	506	831	883	831	426	t0	345	828	771	753	270
t2	203	822	827	807	232	t2	81	882	778	759	82
t6	82	817	855	766	83	t6	31	896	798	757	30
t12	31	802	907	769	30	t12	19	863	737	807	18
t24	19	763	868	769	18	t24	21	869	740	690	21
t48	17	766	855	673	16	t48	24	841	773	647	25
t72	17	667	881	647	19	t72	35	748	727	591	18

Subject 3						Subject 4					
Time (Hr)	EDTA	P800	P700	Linco	Aprotinin	Time (Hr)	EDTA	P800	P700	Linco	Aprotinin
Baseline	4	4	4	4	4	Baseline	4	4	4	4	4
t0	400	623	630	636	406	t0	467	777	773	701	433
t2	121	593	629	646	122	t2	121	796	799	746	168
t6	6	594	671	626	6	t6	7	777	765	715	7
t12	5	607	689	650	5	t12	6	793	771	659	9
t24	6	641	667	665	4	t24	5	759	794	575	6
t48	4	632	667	559	4	t48	5	647	733	489	7
t72	4	581	660	499	11.5	t72	5	607	682	389	7

Baseline active GLP-1 values provide the endogenous concentrations of GLP-1 prior to spiking plasma with a stock concentration of GLP-1.

GLP-1 (Active): (n = 4 Subjects)

Time (Hr)	EDTA	P800	P700	Linco	Aprotinin
Baseline	9	11	11	10	10
t0	430	765	764	730	384
t2	132	773	758	740	151
t6	32	771	772	716	32
t12	15	766	776	721	16
t24	13	758	767	675	12
t48	13	722	757	592	13
t72	15	651	738	532	14

