Measurement of GLP-1 Using the MSD® V-PLEX GLP-1 Active Kit and V-PLEX® GLP-1 Total Kit

White Paper

Introduction

MSD V-PLEX products are developed under rigorous design control, and are analytically validated according to fit-for-purpose principles in accordance with MSD's Quality Management System. In this paper, we discuss the key performance metrics of the new V-PLEX GLP-1 Active and V-PLEX GLP-1 Total Kits, and improvements over the previously designed Active GLP-1 (ver. 2) and Total GLP-1 (ver. 2) Kits.

Comparison of GLP-1 Active Kits

The V-PLEX GLP-1 Active Kit benefits from analytical validation, extensive assay characterization, and lot-to-lot reproducibility that are characteristic of the V-PLEX product line. The antibody pair used in the V-PLEX GLP-1 Active Kit is unchanged from the MSD Active GLP-1 (ver. 2) assay. The specificity of both assays is the result of a highly optimized monoclonal capture antibody specific for the N-terminus cleavage product at the 7 position (see Figure 1). Larger proglucagon protein fragments that are not cleaved at the 7 position of GLP-1 [i.e. MPGF (major proglucagon fragment) or GLP-1 (1–37)] have negligible interference or cross-reactivity in the MSD GLP-1 Active assay. The monoclonal detection antibody is specific to the C-terminus of the active GLP-1 peptide [(7-36) amide and (7-37)] and has excellent specificity for the dominant 36-amide moiety.

The V-PLEX assay incorporates significant improvements to kit configuration and performance. The protocol is simplified with the removal of the blocking step, and the kits include ready-to-use MSD GOLD™ Read Buffer. Lyophilized controls are now available and included in the V-PLEX Plus kit. Additionally, the calibrator was rigorously tested in order to provide the most accurate quantification of active GLP-1 in samples. This results in a 30% differential in concentration measurements between the older assay kit and the new V-PLEX kit. A direct comparison of the two kits shows there is a high degree of concordance between the Active GLP-1 (ver. 2) Kit and the V-PLEX GLP-1 Active Kit (see Figure 2). Table 1 shows the sensitivity and dynamic range of the new kit.

Figure 1. Schematic of the approximate antibody binding sites for the antibody set used in both GLP-1 Active Kits.

	Median LLOD (pM)	LLOD Range (pM)	LLOQ (pM)	ULOQ (pM)
V-PLEX GLP-1 Active Kit	0.020	0.010 – 0.020	0.300	120

Table 1. Sensitivity and dynamic range data for V-PLEX GLP-1 Active Kit.

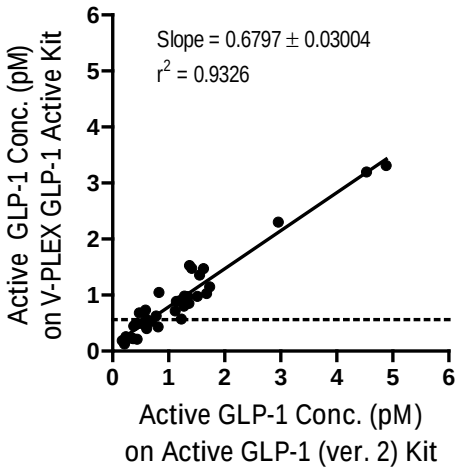

Figure 2. Concordance plot of V-PLEX GLP-1 Active Kit vs. Active GLP-1 (ver. 2) Kit. Twenty-eight human EDTA plasma samples (collected in P800 tubes[1]) were tested on both kits. The slope value of <1 is consistent with the reassignment of the GLP-1 (7–36) amide calibrator concentration in the V-PLEX GLP-1 Active Kit to achieve more accurate measurements.

[1] BD P800 Blood Collection and Preservation System (Product No. 366420 or 366421).

Meso Scale Discovery
1601 Research Boulevard
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Phone: 1-240-314-2600 Fax: 1-240-632-2219

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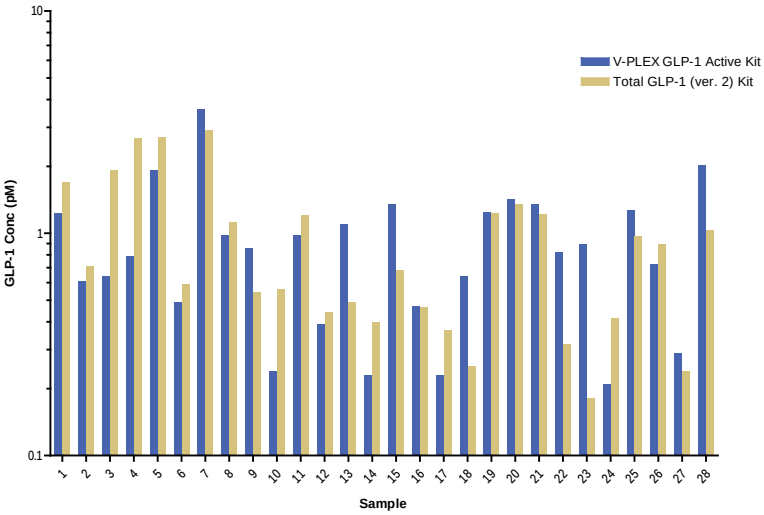
Comparison of GLP-1 Total Kits

It is widely published that the inactive fragment of GLP-1 [(9–36) amide and (9–37)] is the most abundant GLP-1 metabolite in circulation. This is attributed to the rapid degradation of active GLP-1 to the inactive form GLP-1 (9–36) amide by DPP-IV after release from the intestines.[2] This degradation is exceptionally fast and is characterized by the low molar fraction of the active fragment at <15%. A previous version of the GLP-1 Total Kit used a combination of capture antibodies to capture both the active and inactive fragments (see Figure 3). However, it was observed that the assay had a bias toward the active fragment and consequently underrepresented the inactive fragment in samples (see Figure 4). Based on the literature, the total GLP-1 concentration should be significantly higher than the active GLP-1 concentration in normal samples.



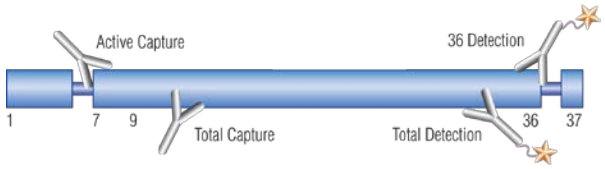


Figure 3 (above). Schematic of the approximate antibody binding sites for the antibody set used in the Total GLP-1 (ver. 2) Kit.

Figure 4 (left). Twenty-eight human EDTA plasma samples (collected in P800 tubes) were tested on both the V-PLEX GLP-1 Active Kit and Total GLP-1 (ver. 2) Kit. The data demonstrate that the active form GLP-1 (7–36) amide is the predominant GLP-1 cleavage product being measured by the Total GLP-1 (ver. 2) Kit.

Redesign of GLP-1 Total Kits

In the redesign of the Total GLP-1 (ver. 2) assay to the V-PLEX GLP-1 Total assay, MSD utilized a monoclonal capture antibody specific for the C-terminus 36amide/37 cleavage point rather than one toward the N-terminus. This distinguishes the V-PLEX GLP-1 Total assay from other MSD GLP-1 assays. The V-PLEX assay achieves specificity solely for GLP-1 sequences characterized by the 36amide/37 cleavage point, regardless of the moiety of the N-terminal cleavage (see Figure 5). This specificity for the GLP-1 fragment reduces significant interference from any circulating MPGF resulting from digestion of proglucagon by the pancreas and is inclusive of the GLP-1 sequence. The V-PLEX assay also uses the GLP-1 (9–36) amide fragment for the calibrator instead of the GLP-1 (7–36) amide fragment used in the Total GLP-1 (ver. 2) Kit. To complete the immunoassay sandwich, MSD utilized a side-viewing antibody, which is known to bind internally to the N-terminus of the inactive GLP-1 (9–36) fragment. Table 2 lists the sensitivity and dynamic range of the V-PLEX GLP-1 Total Kit.

Figure 5. Schematic of the approximate antibody binding sites for the antibody set used in the V-PLEX GLP-1 Total Kit.

	Median LLOD (pM)	LLOD Range (pM)	LLOQ (pM)	ULOQ (pM)
V-PLEX GLP-1 Total Kit	0.017	0.013 – 0.035	0.180	120

Table 2. Sensitivity and dynamic range data for V-PLEX GLP-1 Total Kit.

[2] Holst JJ. The physiology of glucagon-like peptide 1. Physiol. Rev. 2007;87(4):1409-39.

Performance Data of V-PLEX Kits

To assess the performance of the V-PLEX assays, the same 28 human samples of P800-collected EDTA plasma were tested on the V-PLEX GLP-1 Total and V-PLEX GLP-1 Active Kits. The results are in agreement with the expected ratios of active and total GLP-1 reported in the literature (see Figure 6).

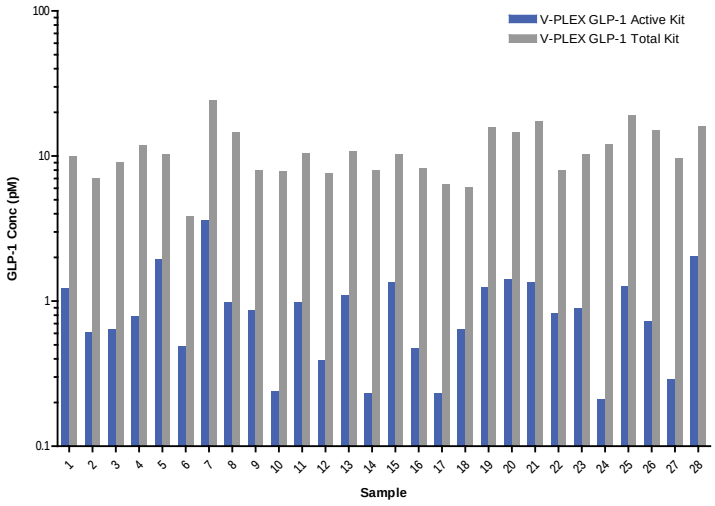


Figure 6. Twenty-eight human EDTA plasma samples (collected in P800 tubes) were tested on both the V-PLEX GLP-1 Active Kit and V-PLEX GLP-1 Total Kit. The active form of GLP-1 [(7–36) amide] is the minority GLP-1 cleavage product measured by the V-PLEX GLP-1 Total Kit.

To further verify the measurement of the two predominant circulating GLP-1 cleavage products, GLP-1 (7–36) amide and GLP-1 (9–36) amide, a 7-point dilution series was prepared using the GLP-1 (7–36) amide or the GLP-1 (9–36) amide peptides. Parallelism and agreement between the curve fits for these two peptides demonstrates their comparable detection in the V-PLEX GLP-1 Total Kit (see Figure 7). Similarly, the reactivity of other GLP-1 cleavage products that are known to be constituents of total GLP-1 was characterized in the assay (see Table 3).

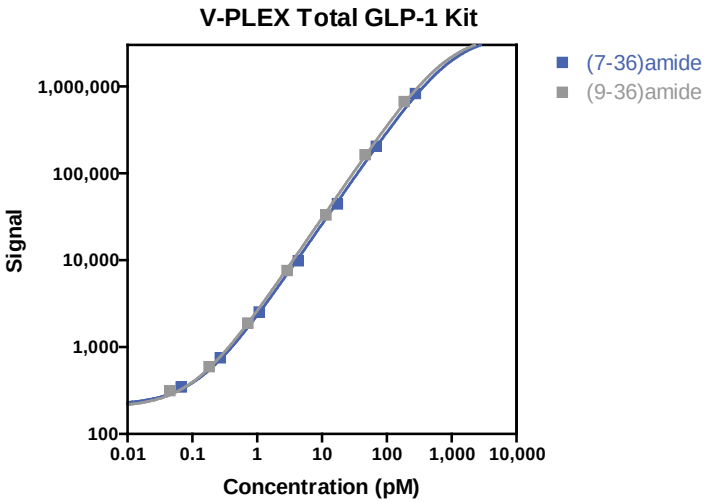


Figure 7. Dilution series of GLP-1 (7–36) amide and GLP-1 (9–36) amide peptides in assay diluent across the dynamic range of the assay (0.017 pM–120 pM).

Peptide	Average % Assay Reactivity	Concentration range tested (pM)
GLP-1 (1-36) amide	87	0.36 - 93
GLP-1 (7-36) amide	87	0.27 - 69
GLP-1 (9-36) amide	100	Calibrator
GLP-1 (7-37)	16	1.8 - 456

Table 3. Detection of four peptides representative of the principal GLP-1 metabolites in circulation in the V-PLEX Total GLP-1 Kit.

Performance of Active and Total GLP-1 Kits in Animal Models

Owing to the sequence homology of GLP-1 across mammalian species, several common animal models were assayed for active GLP-1 and total GLP-1. **Figure** 8 shows that data on mouse, non-human primate (NHP), rat, and canine samples are analogous to that on human samples.

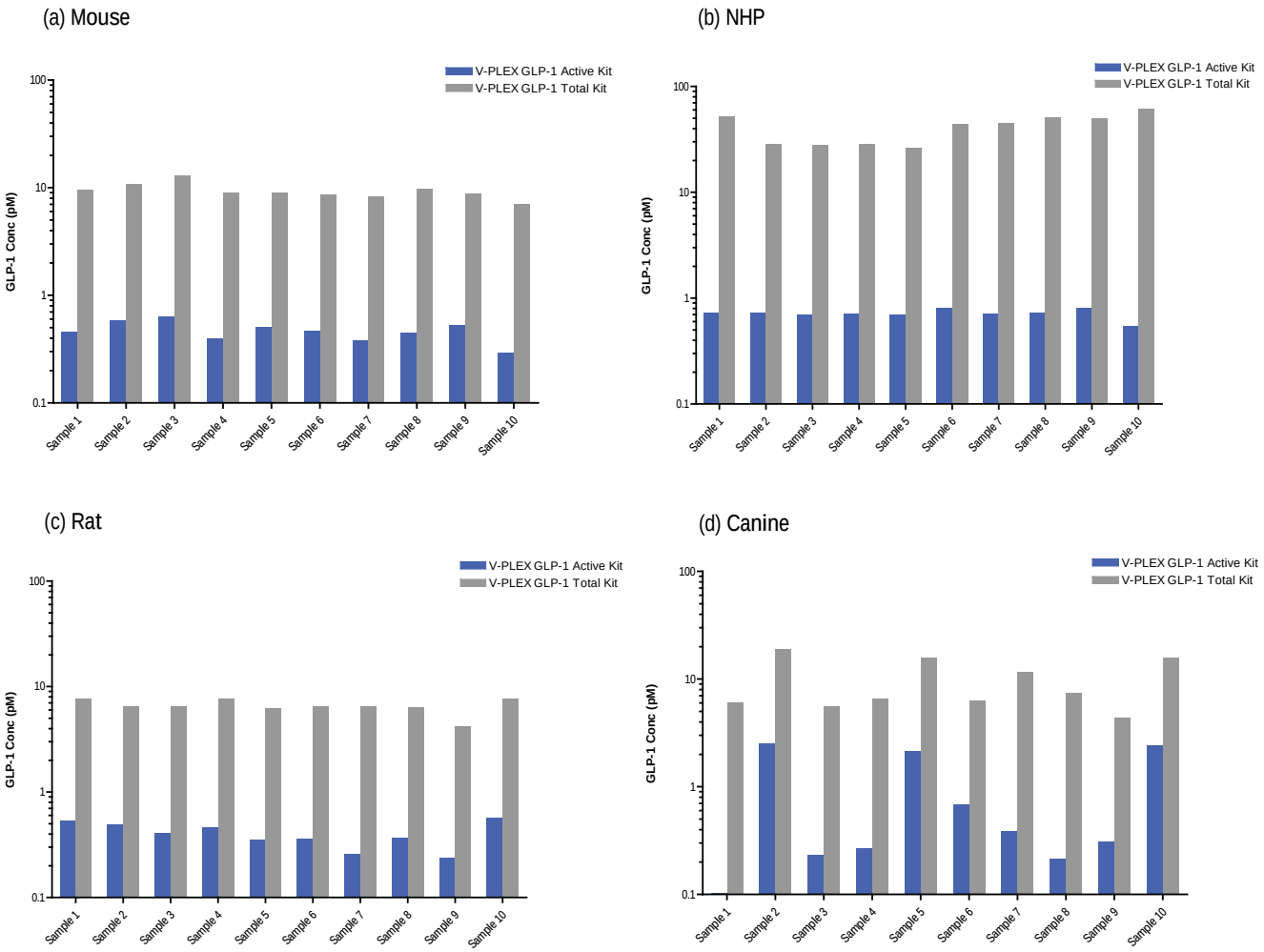


Figure 8. Active GLP-1 and total GLP-1 measurements on: (a) mouse samples collected in EDTA plasma; (b) NHP samples collected in P800 tubes; (c) rat samples collected in P800 tubes; (d) canine samples collected in P800 plasma.

Summary

The V-PLEX GLP-1 Active Kit is an analytically validated and improved version of the GLP-1 Active (ver. 2) Kit with a more accurately assigned lyophilized calibrator and lot-to-lot consistency to ensure continuity throughout research studies. The V-PLEX GLP-1 Total Kit demonstrates high specificity to both the active and inactive fragments of GLP-1, resulting in improved measurement of the total GLP-1 content in samples. Multi-species compatibility for both of these assays facilitates transition between animal models. The V-PLEX GLP-1 Total Kit and V-PLEX GLP-1 Active Kit represent compelling research tools for GLP-1 measurement.