



PlasmaDive™ Reference Peptides Kit

for Human Plasma

MANUAL

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PlasmaDive™ Reference Peptides Kit Panel Components

PlasmaDive™ Reference Peptides Kit	Part No: Ki-3016-48 or Ki-3016-96 Sufficient for analysis of 48 or 96 samples
Reference Peptides Mix	1x or 2x 1.1 ml glass vial, black cap
LC Solution	1x 2 ml tube, clear cap
Dissolution Buffer	1x 2 ml tube, light blue cap
PlasmaDive™ MRM or PRM Panel Plug-ins for SpectroDive™	Available upon request at support@biognosys.com
PlasmaDive™ reference peptides amounts, mass and transition list	Available upon request at support@biognosys.com
PlasmaDive™ Reference Peptide Kit Manual	Available at www.biognosys.com/shop/plasmadive-reference-peptides

Storage and Quality Control of PlasmaDive™ Reference Peptide Kit

Immediately after receiving the kit store:

- ◆ Reference Peptide Mix at **-20°C**
- ◆ All other components should be stored dry at room temperature (15–25°C)

In accordance with **Biognosys'** Quality Management System, each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

Use Limitations

PlasmaDive™ Reference Peptides Kit is intended for mass spectrometry proteomics applications and research use only. This product is not intended for the diagnosis, prevention, or treatment of a disease. All due care and attention should be exercised in the handling of the products.

Product Warranty and Satisfaction Guarantee

Biognosys guarantees the performance of the product when following the instructions and protocols described in this product manual. However, the user must determine the suitability of the product for the intended use. Should the product fail to perform satisfactorily due to any reason other than misuse, Biognosys will replace it free of charge. Biognosys reserves the right to change, alter, or modify any product to enhance its performance and design.

If you have questions about product specifications or performance, please contact us at support@biognosys.com. We also encourage you to contact us if you have any suggestions for improving product performance or for its use in new applications and techniques.

Technical Assistance

Our Technical Department is composed of experienced scientists with extensive practical and theoretical expertise in proteomic technologies and bioinformatics. If you have any questions or experience any difficulties with PlasmaDive™ Reference Peptides Kit please do not hesitate to contact us at support@biognosys.com call +41 44 738 20 40 or visit www.biognosys.com/shop/plasmadive-reference-peptides.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the material safety data sheet (MSDS) available online in convenient and compact PDF format at www.biognosys.com/shop/plasmadive-reference-peptides.

The following risk and safety phrases apply to components of the PlasmaDive™ Reference Peptides Kit:

Dissolution Buffer: Highly flammable liquid and vapour, causes serious eye irritation.

Introduction: The PlasmaDive™ Panel at a Glance

Blood is the most frequently used biological sample in clinical research and routine laboratory diagnostics. Levels of blood proteins reflect the health status of single organs and the body as a whole. Changes in composition of proteins in the blood can be correlated to disease onset or therapy response. Often the changes in protein concentrations relative to each other are the key indicator of a certain condition. Only monitoring the levels of major blood proteins simultaneously makes it possible to recognize the “Big Picture” and explain pathological processes going on in the body.

The PlasmaDive™ Assay Panel was designed for targeted proteomics approaches – Multiple Reaction Monitoring (MRM, also called SRM) and Parallel Reaction Monitoring (PRM) – which focus on quantifying predefined sets of proteins with high sensitivity and reproducibility. Biognosys is unique in its ability to perform scheduled highly multiplexed MRM and PRM measurements based on its innovative iRT concept and specifically developed the MRM and PRM signal processing software – SpectroDive™. The PlasmaDive™ kit includes a reference peptide mix that allows users to measure absolute concentrations of the target proteins.

The PlasmaDive™ Panel optimally combines 100 peptide MRM or PRM assays in one scheduled multiplexed method. Each peptide is representative of a human plasma protein (**Table 1**). The PlasmaDive™ protocol requires as little as 10 µl of plasma and 48 hours to provide to provide actionable clinically-relevant data. The panel is therefore a perfect solution for large-scale experiments requiring high throughput.

The complete PlasmaDive™ workflow was designed to be an end-to-end solution, from a plasma sample to actionable results. While the PlasmaDive™ Reference Peptide Mix is a key component, the full workflow additionally includes Sample Preparation Kit Pro (Ki-3013) for reproducible proteomic sample processing in a high throughput fashion and SpectroDive™ (Sw-3002), Biognosys’ proprietary software for automated SRM/MRM and PRM method set-up, signal processing and experiment analysis. Visit www.biognosys.com/shop/ for additional information or www.biognosys.com/shop/plasmadive for an in-depth look into the complete PlasmaDive™ workflow.

Table 1. List of proteins quantified with PlasmaDive™ Panel

UniPot ID	Entry Name	Protein Name
P02763	A1AG1_HUMAN	Alpha-1-acid glycoprotein 1 (Orosomucoid-1)
P19652	A1AG2_HUMAN	Alpha-1-acid glycoprotein 2 (Orosomucoid-2)
P01009	A1AT_HUMAN	Alpha-1-antitrypsin
P04217	A1BG_HUMAN	Alpha-1B-glycoprotein
P08697	A2AP_HUMAN	Alpha-2-antiplasmin (Serpin F2)
P02750	A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein (LRG)
P01023	A2MG_HUMAN	Alpha-2-macroglobulin (Alpha-2-M)
P01011	AACT_HUMAN	Alpha-1-antichymotrypsin (ACT)
P43652	AFAM_HUMAN	Afamin (Alpha-albumin)
P02768	ALBU_HUMAN	Serum albumin

P35858	ALS_HUMAN	Insulin-like growth factor-binding protein complex acid labile subunit
P02760	AMBP_HUMAN	Protein AMBP
P01019	ANGT_HUMAN	Angiotensinogen (Serpins A8)
P01008	ANT3_HUMAN	Antithrombin-III (Serpins C1)
P02647	APOA1_HUMAN	Apolipoprotein A-I
P02652	APOA2_HUMAN	Apolipoprotein A-II
P06727	APOA4_HUMAN	Apolipoprotein A-IV
P04114	APOB_HUMAN	Apolipoprotein B-100
P02654	APOC1_HUMAN	Apolipoprotein C-I
P02655	APOC2_HUMAN	Apolipoprotein C-II
P02656	APOC3_HUMAN	Apolipoprotein C-III
P05090	APOD_HUMAN	Apolipoprotein D
P02649	APOE_HUMAN	Apolipoprotein E
P02749	APOH_HUMAN	Apolipoprotein H
O14791	APOL1_HUMAN	Apolipoprotein L1
O95445	APOM_HUMAN	Apolipoprotein M
P43251	BTD_HUMAN	Biotinidase
P02745	C1QA_HUMAN	Complement C1q subcomponent subunit A
P02746	C1QB_HUMAN	Complement C1q subcomponent subunit B
P02747	C1QC_HUMAN	Complement C1q subcomponent subunit C
P00736	C1R_HUMAN	Complement C1r subcomponent
P09871	C1S_HUMAN	Complement C1s subcomponent
P04003	C4BPA_HUMAN	C4b-binding protein alpha chain
P08185	CBG_HUMAN	Corticosteroid-binding globulin (Serpins A6)
O43866	CD5L_HUMAN	CD5 antigen-like (CT-2) (SP-alpha)
P00450	CERU_HUMAN	Ceruloplasmin (Ferroxidase)
P00751	CFAB_HUMAN	Complement factor B
P08603	CFAH_HUMAN	Complement factor H (H factor 1)
P05156	CFAI_HUMAN	Complement factor I
P06276	CHLE_HUMAN	Cholinesterase (EC 3.1.1.8)
P10909	CLUS_HUMAN	Clusterin (Aging-associated gene 4 protein) (Apolipoprotein J)
P06681	CO2_HUMAN	Complement C2
P01024	CO3_HUMAN	Complement C3
POC0L4	CO4A_HUMAN	Complement C4-A
P01031	CO5_HUMAN	Complement C5
P07357	CO8A_HUMAN	Complement component C8 alpha chain
P02748	CO9_HUMAN	Complement component C9
P02775	CXCL7_HUMAN	Platelet basic protein (C-X-C motif chemokine 7)
P00488	F13A_HUMAN	Coagulation factor XIII A chain
P05160	F13B_HUMAN	Coagulation factor XIII B chain
P00742	FA10_HUMAN	Coagulation factor X
P00740	FA9_HUMAN	Coagulation factor IX
P23142	FBLN1_HUMAN	Fibulin-1
P02765	FETUA_HUMAN	Alpha-2-HS-glycoprotein
Q9UGM5	FETUB_HUMAN	Fetuin-B
P02671	FIBA_HUMAN	Fibrinogen alpha chain
P02679	FIBG_HUMAN	Fibrinogen gamma chain
P02751	FINC_HUMAN	Fibronectin (FN)

P06396	GELS_HUMAN	Gelsolin (Actin-depolymerizing factor)
P22352	GPX3_HUMAN	Glutathione peroxidase 3
P68871	HBB_HUMAN	Hemoglobin subunit beta
P02042	HBD_HUMAN	Hemoglobin subunit delta (Delta-globin)
P02790	HEMO_HUMAN	Hemopexin (Beta-1B-glycoprotein)
P05546	HEP2_HUMAN	Heparin cofactor 2
P00738	HPT_HUMAN	Haptoglobin
P00739	HPTR_HUMAN	Haptoglobin-related protein
P04196	HRG_HUMAN	Histidine-rich glycoprotein
P05155	IC1_HUMAN	Plasma protease C1 inhibitor
P01876	IGHA1_HUMAN	Ig alpha-1 chain C region
P01877	IGHA2_HUMAN	Ig alpha-2 chain C region
P01857	IGHG1_HUMAN	Ig gamma-1 chain C region
P01859	IGHG2_HUMAN	Ig gamma-2 chain C region
P01860	IGHG3_HUMAN	Ig gamma-3 chain C region (HDC)
P01871	IGHM_HUMAN	Ig mu chain C region
P05154	IPSP_HUMAN	Plasma serine protease inhibitor
P19827	ITIH1_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H1
P19823	ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2
Q14624	ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4
P29622	KAIN_HUMAN	Kallistatin (Kallikrein inhibitor)
P03952	KLKB1_HUMAN	Plasma kallikrein
P01042	KNG1_HUMAN	Kininogen-1
P36955	PEDF_HUMAN	Pigment epithelium-derived factor (PEDF)
Q96PD5	PGRP2_HUMAN	N-acetylmuramoyl-L-alanine amidase
P02776	PLF4_HUMAN	Platelet factor 4 (Oncostatin-A)
P00747	PLMN_HUMAN	Plasminogen
P27169	PON1_HUMAN	Serum paraoxonase
Q92954	PRG4_HUMAN	Proteoglycan 4
P02753	RET4_HUMAN	Retinol-binding protein 4
P35542	SAA4_HUMAN	Serum amyloid A-4 protein
P49908	SEPP1_HUMAN	Selenoprotein P
P04278	SHBG_HUMAN	Sex hormone-binding globulin
P05452	TETN_HUMAN	Tetranectin
P05543	THBG_HUMAN	Thyroxine-binding globulin (Serpins A7)
P00734	THRB_HUMAN	Prothrombin
P02787	TRFE_HUMAN	Serotransferrin
P02766	TTHY_HUMAN	Transthyretin
P02774	VTDB_HUMAN	Vitamin D-binding protein
P04004	VTNC_HUMAN	Vitronectin
P04275	VWF_HUMAN	von Willebrand factor
P25311	ZA2G_HUMAN	Zinc-alpha-2-glycoprotein

Important Notes before Starting

Before starting with the sample preparation, read through the steps carefully and make sure all the required reagents and equipment are available.

The kit components are sufficient for 48 (Ki-3016-48) or 96 (Ki-3016-96) human plasma samples.

The PlasmaDive™ Reference Peptides Kit does contain only reference peptide mix and buffers to solubilize these. All aspects (buffers or protocols) of sample processing, method set-up and data analysis are NOT covered in the scope of this kit. If you need information on these subjects, please refer to the PlasmaDive™ complete workflow (www.biognosys.com/shop/plasmadive), which includes Sample Preparation Kit Pro (www.biognosys.com/shop/sample-preparation-kit-pro) and SpectroDive™ (www.biognosys.com/shop/spectrodive) or send an inquiry to support@biognosys.com.

Additionally Required Laboratory Equipment and Consumables

Single channel pipettes (0.5 µl – 1000 µl) with corresponding tips

Vortex mixer

Optional: Ultra-sonication device

LC-MS vials

Reference Peptide Kit Usage and Recommended Sample Preparation

1. Prepare **Reference Peptide Mix** by adding to the glass vial 20 µl of **Dissolution Buffer**.
2. Vortex briefly.
3. Add 100 µl of **LC Solution** to the **Reference Peptide Mix**.
4. Vortex the **Reference Peptide Mix** thoroughly.

Optional: Sonicate for 5 min

Recommended spike-in amounts to be compatible with the PlasmaDive™ workflow including the automated analysis with SpectroDive™:

1. Dissolve dried or dilute ready-to-inject processed plasma samples to a final concentration of 0.44 µg/µl with an LC-MS compatible buffer (*not included in this kit*).
2. Vortex or, if possible, sonicate the samples.

3. Centrifuge the dissolved samples at 4°C and maximum speed for 20 min.
4. Transfer 6 µl of sample supernatants to LC-MS vials; store remaining samples at -20°C for future analysis.
5. Add 2 µl/sample of **Reference Peptide Mix** to each LC-MS vial.
6. Inject 3 µl/sample for all LC-MS measurements (corresponds to about 1 µg of sample peptides per injection)

For the detailed full PlasmaDive™ workflow visit

www.biognosys.com/shop/plasmadive.

If you require additional information to set-up your method of choice with PlasmaDive™ Reference Peptides (e.g. peptide amounts, mass list, transition list), please send an inquiry to support@biognosys.com.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving issues that may arise.

Issue	Possible Cause	Recommended Solution
The first iRT peptide behaves irregularly	LC gradient starts with > 1% AcN	Because of the high peptide hydrophilicity slight irregularities of the first iRT peptide behaviour still can be observed. This usually does not influence the quality of calibration.
iRT calibration is not linear	Non-linear gradient was used	Use linear gradient and LC settings
	A minor issue in chromatographic system if there are only 1 or 2 outliers from linearity	Check manually if respective iRT peaks are correctly integrated and if the peak shapes are satisfactory. If not, exclude outliers from calibration by clicking on "Edit..." in '2) Choose LC calibration' section.
	A major defect in chromatographic system if iRT calibration is not linear through the whole range	Please contact the technical support of the LC manufacturer.

Issue	Possible Cause	Recommended Solution
No signal on LC-MS when analyzing samples	Problem with sample preparation	Prepare and analyze a control sample with Reference Peptide Mix diluted in LC Buffer (keep the dilution rate this protocol). If you see a signal on LC-MS your initial samples were not prepared correctly.
	Problem with the LC-MS system	If you don't see any signal on your LC-MS after analyzing the control sample from above please contact the technical support of your LC-MS vendor.

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Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of PlasmaDive™ Panel to the following terms:

1. PlasmaDive™ Reference Peptide Kit may be used solely in accordance with the PlasmaDive™ Reference Peptide Kit Manual and with components contained in the panel only. Biognosys grants no license under any of its intellectual property to use or incorporate the enclosed components of this assay panel with any components not included within this panel except as described in the PlasmaDive™ Reference Peptide Kit Manual and additional protocols available at www.biognosys.com/shop/plasmadive-reference-peptides.
2. Other than expressly stated licenses, Biognosys makes no warranty that this panel and/or its use(s) do not infringe the rights of third-parties.
3. The PlasmaDive™ Reference Peptide Kit and its components are licensed for 48 (Ki-3016-48) or 96 (Ki-3016-48) samples (one-time use) and may not be reused, refurbished, or resold.
4. Biognosys specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of this panel agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. Biognosys may enforce the prohibitions of this Limited License Agreement in any court, and shall recover all its investigative and court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

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