



PlasmaDeepDive™ Reference Peptides Kit

for Depleted Human Plasma

MANUAL

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PlasmaDeepDive™ Reference Peptides Kit Components

PlasmaDeepDive™ Reference Peptides Kit	Part No: Ki-3017-48 or Ki-3017-96 Sufficient for analysis of 48 or 96 samples
Reference Peptides Mix	1x or 2x 1.1 ml glass vial, dark blue cap
LC Solution	1x 2 ml tube, clear cap
Dissolution Buffer	1x 2 ml tube, light blue cap
PlasmaDeepDive™ MRM and PRM Panel plug-ins for SpectroDive™	Available upon request at support@biognosys.com
PlasmaDeepDive™ reference peptides amounts, mass and transition list	Available upon request at support@biognosys.com
PlasmaDeepDive™ Assay Panel Manual	Available at www.biognosys.com/shop/plasmadeepdive-reference-peptides

Storage and Quality Control of the **PlasmaDeepDive™** Reference Peptides 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

PlasmaDeepDive™ Reference Peptides Kit is intended for mass spectrometry proteomics applications and research use only. This product is not intended for the diagnostic, 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 its particular 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 PlasmaDeepDive™ 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/plasmadeepdive-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/plasmadeepdive-reference-peptides.

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

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

Introduction: PlasmaDeepDive™ Panel at a Glance

Blood is the most frequently used human biological sample in research and routine laboratory diagnostics. Protein levels in blood plasma reflect the health status of single organs as well as the body as a whole. Changes in blood protein levels can be directly correlated to a disease onset or therapy response and are often key indicators of a certain physiological or pathological condition. However, to evaluate the overall status of the body simultaneous monitoring of levels of major blood proteins is required. PlasmaDeepDive™ Assay Panel is specifically designed for this task enabling quantification of up to 100 plasma proteins in a single sample with unbeatable specificity and precision. It sets a new standard for clinical research, biomarker screening and development of companion diagnostics.

The PlasmaDeepDive™ Assay Panel was designed for targeted proteomics approaches - Multiple Reaction Monitoring (MRM, also called SRM) or 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 signal processing software – SpectroDive™. Both the iRT and SpectroDive™ are integral parts of Biognosys' Assay Panels.

PlasmaDeepDive™ Multiplexed Assay Panel optimally combines 100 peptide MRM or PRM assays in one scheduled method. Each peptide is representative of a human plasma protein (**Table 1**). Since the PlasmaDeepDive™ Assay Panel is designed for depleted plasma samples, 16 peptides corresponding to the typically depleted proteins are included into the panel to monitor the depletion quality (Group "Depleted Protein"). Furthermore, 9 housekeeping proteins (Group "QC Protein") are measured in the panel to allow for normalization and consequently higher reproducibility of the results. Other 75 proteins in the panel represent relevant targets for biomarker screening (Group "Target").

The complete PlasmaDeepDive™ workflow was designed to be an end-to-end solution, from a depleted plasma sample to actionable results. While the PlasmaDeepDive™ 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/plasmadeepdive for an in-depth look into the complete PlasmaDeepDive™ workflow.

Table 1. List of proteins quantified with PlasmaDeepDive™ Assay Panel

UniPot ID	Protein Name	Group
P11021	78 kDa glucose-regulated protein	Target

Q15848	Adiponectin	Target
Q96KN2	Beta-Ala-His dipeptidase	Target
P01138	Beta-nerve growth factor	Target
P23560	Brain-derived neurotrophic factor	Target
P55290	Cadherin-13	Target
P33151	Cadherin-5 (CD144)	Target
Q961Y4	Carboxypeptidase B2	Target
Q9NQ79	Cartilage acidic protein 1	Target
P04637	Cellular tumor antigen p53	Target
P36222	Chitinase-3-like protein 1	Target
P26441	Ciliary neurotrophic factor	Target
P03951	Coagulation factor XI	Target
Q9NZP8	Complement C1r subcomponent-like protein	Target
P0C0L5	Complement C4-B	Target
P06850	Corticoliberin	Target
P01034	Cystatin-C	Target
Q01459	Di-N-acetylchitobiase	Target
P09172	Dopamine beta-hydroxylase	Target
Q12805	EGF-containing fibulin-like extracellular matrix protein 1	Target
P00533	Epidermal growth factor receptor	Target
P09104	Gamma-enolase	Target
P14136	Glial fibrillary acidic protein	Target
O43301	Heat shock 70 kDa protein 12A	Target
Q0VDF9	Heat shock 70 kDa protein 14	Target
P08107	Heat shock 70 kDa protein 1A/1B	Target
P34931	Heat shock 70 kDa protein 1-like	Target
P34932	Heat shock 70 kDa protein 4	Target
O95757	Heat shock 70 kDa protein 4L	Target
P17066	Heat shock 70 kDa protein 6	Target
P11142	Heat shock cognate 71 kDa protein	Target
Q04756	Hepatocyte growth factor activator	Target
P05019	Insulin-like growth factor I	Target
P01344	Insulin-like growth factor II	Target
P18065	Insulin-like growth factor-binding protein 2	Target
P17936	Insulin-like growth factor-binding protein 3	Target
P35858	Insulin-like growth factor-binding protein complex acid labile subunit	Target
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3	Target
Q9NPH3	Interleukin-1 receptor accessory protein	Target
P22301	Interleukin-10	Target
O75874	Isocitrate dehydrogenase [NADP] cytoplasmic	Target
P41159	Leptin	Target
P18428	Lipopolysaccharide-binding protein	Target
P07195	L-lactate dehydrogenase B chain	Target
P14151	L-selectin (CD62L)	Target
P51884	Lumican	Target
P14780	Matrix metalloproteinase-9	Target
P01033	Metalloproteinase inhibitor 1	Target
P16455	Methylated-DNA--protein-cysteine methyltransferase	Target
P08571	Monocyte differentiation antigen CD14	Target

Q13201	Multimerin-1	Target
Q9UNW1	Multiple inositol polyphosphate phosphatase 1	Target
P20929	Nebulin	Target
P48681	Nestin	Target
P13591	Neural cell adhesion molecule 1 (CD56)	Target
O00533	Neural cell adhesion molecule L1-like protein	Target
P08949	Neuromedin-B	Target
O14786	Neuropilin-1 (CD304)	Target
P30990	Neurotensin/neuromedin N	Target
P60484	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	Target
P80108	Phosphatidylinositol-glycan-specific phospholipase D	Target
P02775	Platelet basic protein	Target
P07359	Platelet glycoprotein Ib alpha chain	Target
P40197	Platelet glycoprotein V (CD42d)	Target
P20742	Pregnancy zone protein	Target
P01303	Pro-neuropeptide Y	Target
P20366	Protachykinin-1	Target
Q9HD89	Resistin	Target
P02743	Serum amyloid P-component	Target
P38646	Stress-70 protein, mitochondrial	Target
Q16563	Synaptophysin-like protein 1	Target
P07996	Thrombospondin-1	Target
Q6EMK4	Vasorin	Target
P22891	Vitamin K-dependent protein Z	Target
P04275	von Willebrand factor	Target
P01009	Alpha-1-antitrypsin	Depleted Protein
P01023	Alpha-2-macroglobulin	Depleted Protein
P02647	Apolipoprotein A-I	Depleted Protein
P02652	Apolipoprotein A-II	Depleted Protein
P01024	Complement C3	Depleted Protein
P02671	Fibrinogen alpha chain	Depleted Protein
P02675	Fibrinogen beta chain	Depleted Protein
P02679	Fibrinogen gamma chain	Depleted Protein
P00738	Haptoglobin	Depleted Protein
P01876	Ig alpha-1 chain C region	Depleted Protein
P01857	Ig gamma-1 chain C region	Depleted Protein
P01859	Ig gamma-2 chain C region	Depleted Protein
P01871	Ig mu chain C region	Depleted Protein
P02787	Serotransferrin (Transferrin)	Depleted Protein
P02768	Serum albumin	Depleted Protein
P02766	Transthyretin	Depleted Protein
P04217	Alpha-1B-glycoprotein	QC protein
P00450	Ceruloplasmin	QC protein
P02790	Hemopexin	QC protein
P19823	Inter-alpha-trypsin inhibitor heavy chain H2	QC protein
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	QC protein
P01042	Kininogen-1	QC protein
P05155	Plasma protease C1 inhibitor	QC protein
P02774	Vitamin D-binding protein	QC protein

P04004

Vitronectin

QC protein

Important Notes before Start

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-3017-48) or 96 (Ki-3017-96) depleted human plasma samples.

The PlasmaDeepDive™ Reference Peptides Kit does contain only reference peptides 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 PlasmaDeepDive™ complete workflow (<http://www.biognosys.com/shop/plasmadeepdive>), 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 PlasmaDeepDive™ workflow including the automated analysis with SpectroDive™:

1. Dissolve dried or dilute ready-to-inject processed depleted plasma samples to a final concentration of 0.88 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 2 µg of sample peptides per injection).

For the detailed full PlasmaDeepDive™ workflow visit

www.biognosys.com/shop/plasmadeepdive.

If you require additional information to set-up your method of choice with PlasmaDeepDive™ 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
LC column is clogged during sample analysis	Solid particles were not completely removed from the samples during the centrifugation (step 3)	Transfer final samples to single vials and repeat a cold centrifugation (4°C) as described in step 3.
The first iRT peptide behaves irregularly	LC gradient starts with > 5% AcN	Ensure you use the LC settings as recommended in Section Error! Reference source not found. 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 in LC settings

Issue	Possible Cause	Recommended Solution
	A minor issue in chromatographic system if there are only 1 or 2 outliers from linearity	Check manually if iRT peaks are correctly integrated and if the peak shapes are satisfactory. If yes, exclude outliers from calibration.
	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.
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 of the 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 PlasmaDeepDive™ Reference Peptide Kit to the following terms:

1. PlasmaDeepDive™ Reference Peptide Kit may be used solely in accordance with the PlasmaDeepDive™ Assay Panel 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 assay panel except as described in the PlasmaDeepDive™ Reference Peptides Kit Manual and additional protocols available at www.biognosys.com/shop/plasmadeepdive-reference-peptides.
2. Other than expressly stated licenses, Biognosys makes no warranty that this assay panel and/or its use(s) do not infringe the rights of third-parties.
3. The PlasmaDeepDive™ Reference Peptide Kit and its components are licensed for (Ki-3017-48) or 96 (Ki-3017-96) 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 assay 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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