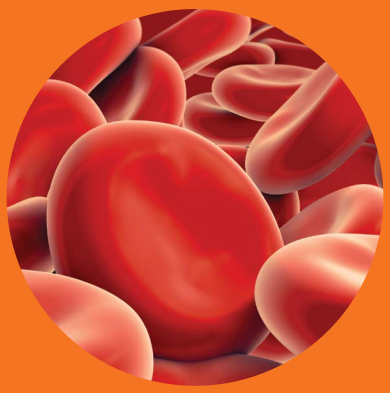
BD™ P100 Blood Collection System
for Plasma Protein Preservation

Instant Stabilisation
at point of collection



Proteins

Enabling Reproducible Protein Biomarker Preservation

BD

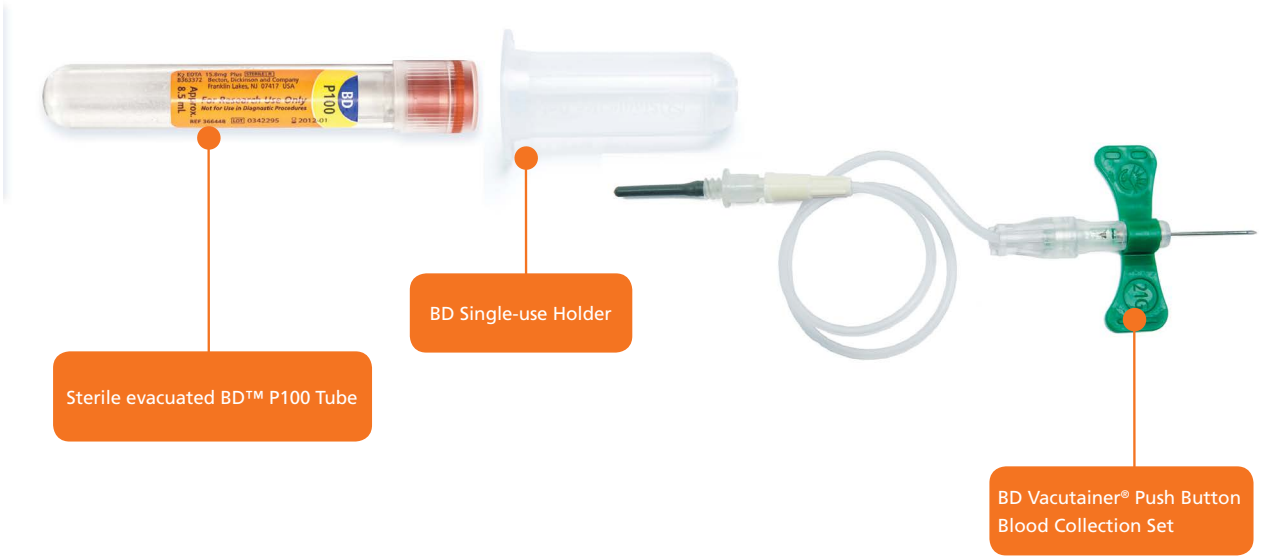
Helping all people
live healthy lives

Minimise Preanalytical Variability with the BD™ P100 Blood Collection System for Plasma Protein Preservation

Human plasma contains a wide variety of proteins that hold potential as biomarkers for diagnostic assays. These proteins are subject to proteolytic degradation and modification during and after blood collection, impeding their discovery and validation.

The BD™ P100 Blood Collection System minimises blood plasma protein preanalytical variability by standardising collection and transportation of specimens, to improve protein analysis.

BD™ P100 Blood Collection System



The BD™ Vacutainer Blood Collection system comprises a choice of safety blood collection devices, holder and BD™ P100 Tube.

1) High Quality Plasma

The BD™ P100 Tube is spray coated with K_2EDTA anticoagulant to ensure a consistent quantity of additive particles on the interior wall of the tube that will quickly and consistently dissolve when blood enters the tube and is mixed.

2) Stabilised Proteins

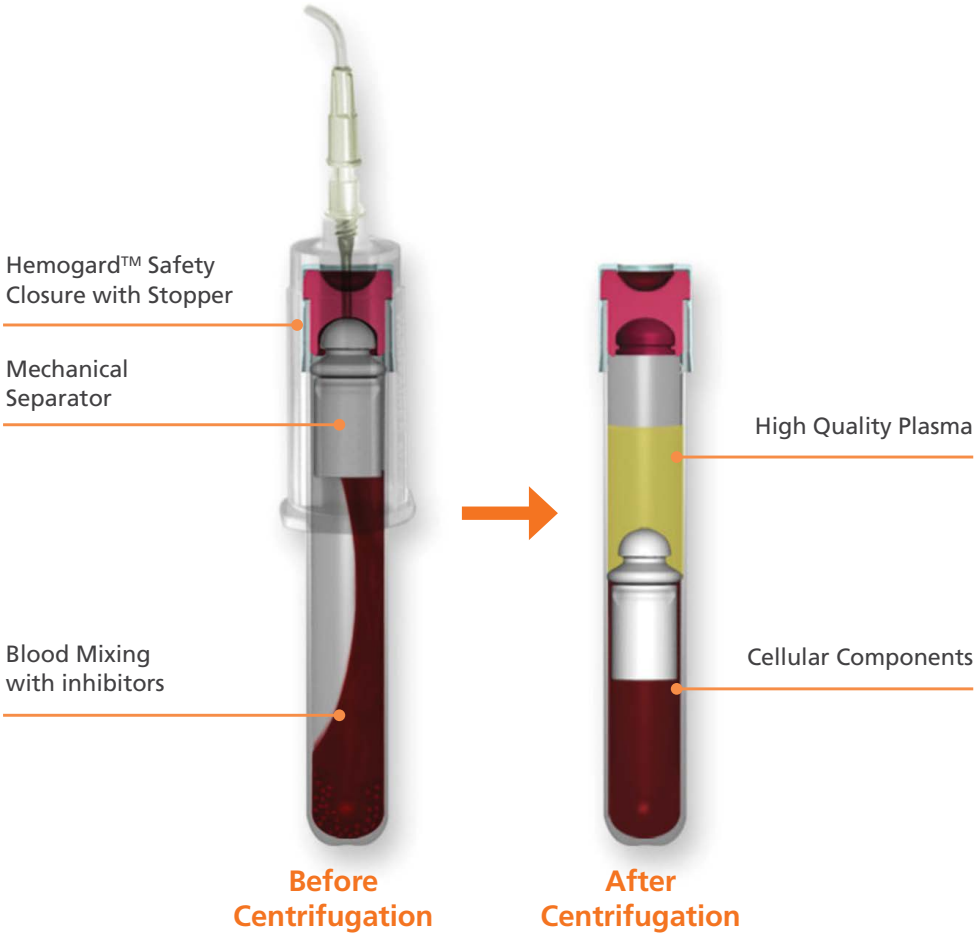
The tube also contains a spray coated proprietary protease inhibitor cocktail, specifically formulated for stabilisation of human plasma proteins at the point of collection, to ensure minimal proteolytic degradation of proteins in blood.

3) Minimal cellular contamination

Contamination of plasma samples by the cellular elements of blood, such as platelets, can significantly alter the detectable protein content of the sample. Inclusion of an innovative mechanical separator within BD™ P100 Tubes provides a superior plasma separation compared to gel based separation technologies to further improve plasma protein stabilisation.

Disclosure: *BD™ P100 is for research use only – not for use in diagnostic procedures*

BD™ P100 Blood Collection Tube



BD™ P100 Blood Collection System enables

- On-board protein stabilisers with Point-of-Collection protection of plasma proteins.
- Innovative mechanical separator minimising cellular contamination.
- No contamination of the collected protein plasma sample using the closed, sterile BD Vacutainer™ Blood Collection System.
- Reproducible blood draw from the BD Vacutainer™ Tube predetermined vacuum ensure optimal analysis.
- Potential elimination of secondary sample transfer steps with plasma proteins being stabilised and transported within the BD™ P100 Tube.
- Optimal User protection from patient blood with BD Hemogard™ safety closure and plastic blood collection tube.

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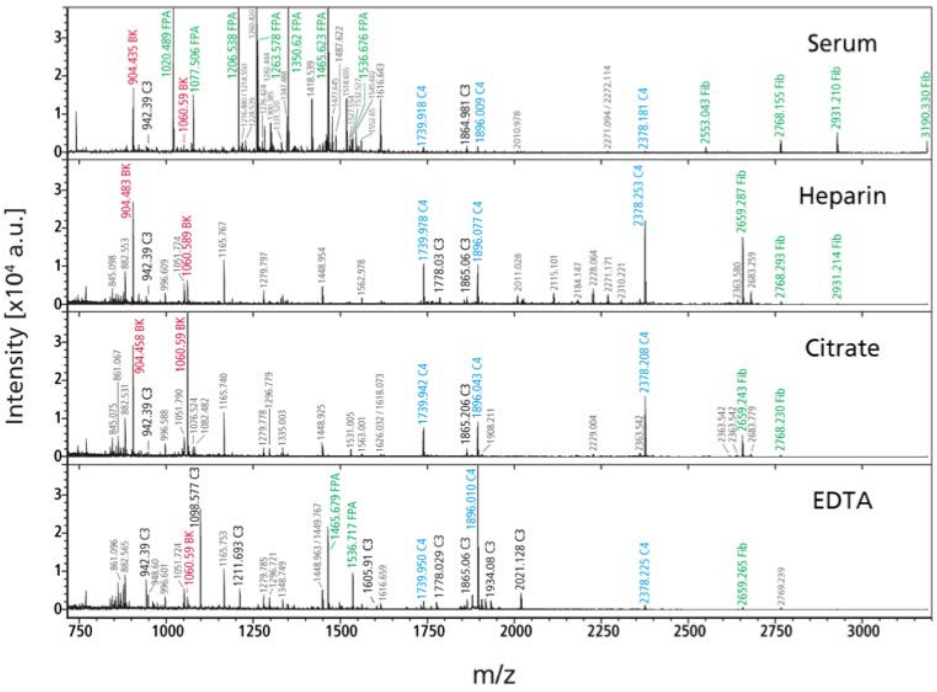
Why is EDTA plasma preferable for blood protein analysis?

It is generally accepted that protein stability in blood increases according to the following preparation techniques - serum, heparin, citrate, EDTA and BD™ P100 Tube[1].

The comparison of blood drawn from a single patient into a series of BD Vacutainer® Blood Collection Tubes for mass spectrometry analysis confirms EDTA plasma provides the best plasma protein for analysis[2].



Intrinsic serum proteases generate peptides by *ex-vivo* digestions of proteins during the clotting process, which results in the substantial peptide difference between a serum and a plasma sample. Anticoagulants have an inherent protease inhibition and peptide variability is muted in plasma versus serum, supporting previous recommendations that plasma is the preferred sample for the analysis of blood proteins.

The heparin and citrate blood plasmas are often similar in peptide content by peak positions. When compared with the heparin and citrate samples, the EDTA blood plasma displays more consistent peaks, thus making it preferable for the analysis of proteins.

Intrinsic protease and peptidase actions occur during the first minutes of blood collection, which accounts for the peptide differences observed with serum and the three anticoagulated plasma samples (EDTA, citrate, and heparin). Despite the fact that the anticoagulants provide a partial inhibitory effect, residual plasma protease and peptidase activities still result in time-dependent variations and instability of the samples including EDTA.

References:

1. Omenn GS et al, Overview of the HUPO Plasma Proteome Project: Results from the pilot phase with 35 collaborating laboratories and multiple analytical groups generating a core dataset of 3020 proteins and a publicly-available database, proteomics, 13, 3226-45, 2005.
2. Jizu Yi, Zhaoxia Liu, David Craft, Patrick O'Mullan, Gang Ju, and Craig A. Gelfand, Intrinsic Peptidase Activity Causes a Sequential Multi-Step Reaction (SMSR) in Digestion of Human Plasma Peptides, Journal of Proteome Research, Vol. 7, 5112–5118, 2008.

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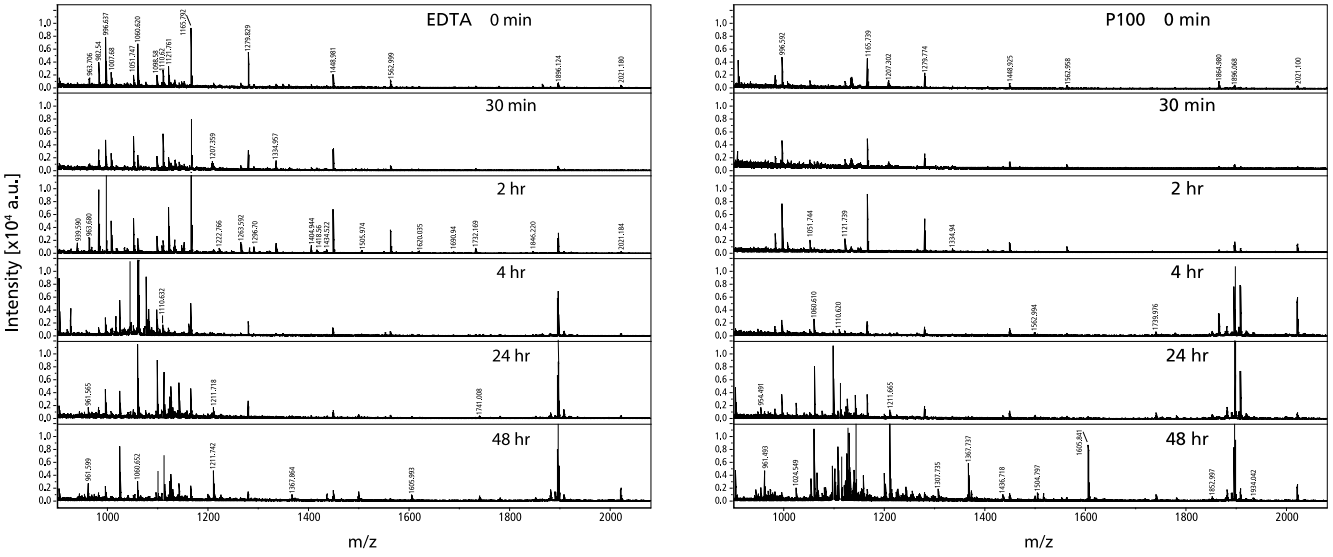
Protease inhibitors improve blood protein plasma stability

Protease inhibitors included in BD™ P100 Tube modulate and suppress intrinsic protease and peptidase activities at the point of blood collection, providing the best source of plasma protein for biomarker discovery and clinical research.



The comparison of the protein stability in EDTA plasma and BD™ P100 Tubes shows improved stability of proteins at the time of blood collection. The results in the figure above show immediate changes in detected protein at the time of blood collection in EDTA samples and significant degradation appears after 30 minutes. The peak changes seen in the BD™ P100 samples take place at a much slower rate compared to EDTA samples, ensuring protein stability at room temperature for most proteins for up to 4 hours [3,4].

References:

3. Jizu Yi, Changki Kim, and Craig A. Gelfand, Inhibition of Intrinsic Proteolytic Activities Moderates Preanalytical Variability and Instability of Human Plasma, Journal of Proteome Research, Vol. 6, 1768-1781, 2007.
4. David Craft, Jizu Yi and Craig A. Gelfand, Time-Dependent and Sample-to-Sample Variations in Human Plasma Peptidome are Both Minimised Through Use of Protease Inhibitor, Analytical Letters, 42, 1398–1406, 2009.

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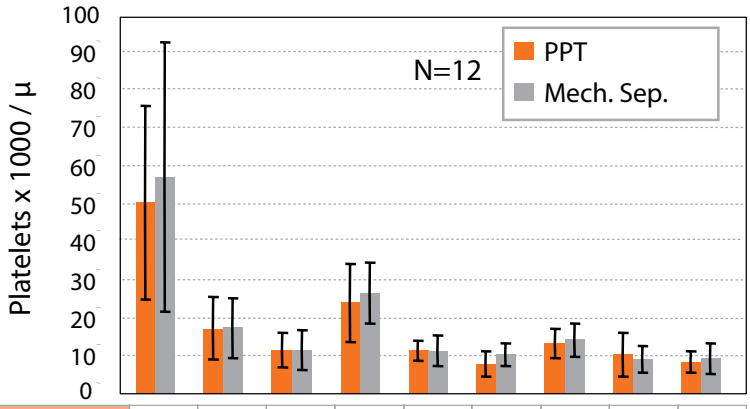
The mechanical separator improves plasma purity

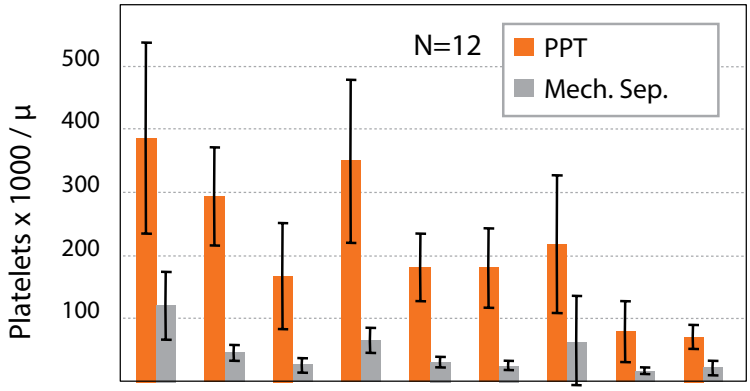
To further improve the stability of the BD™ P100 Tube an innovative 'mechanical' separator is used to provide an improved separation of cellular components from the plasma than that found in existing gel based separators.

Comparing the BD™ P100 Tube with the BD™ PPT (Plasma Preparation Tube), a gel based separator tube, demonstrates the improved separation of cellular components from plasma. The comparison of platelet contamination between the BD™ P100 and BD™ PPT Tubes by measuring the number of platelets directly after centrifugation of collected blood and then again after mixing the tubes demonstrated the improved separation of cellular components of blood using the BD™ P100 Tube[5].



Directly Post-centrifugation

Centrifuge time, min	10	20	30	10	20	30	10	20	30
Centrifuge RCF	1100			1600			2500		



Mixing after centrifugation

Centrifuge time, min	10	20	30	10	20	30	10	20	30
Centrifuge RCF	1100			1600			2500		

The results clearly show the increased effectiveness of the BD™ P100 Tube mechanical separator to provide a better separation of cellular components from the plasma than existing gel based separators for plasma analysis.

References:

5. Craig A. Gelfand, Jizu Yi, David Warunek, David Craft, and Patrick O'Mullan, Preanalytical variability of plasma samples, and mitigating strategies – BD Poster, HUPO, Seatle, 2007

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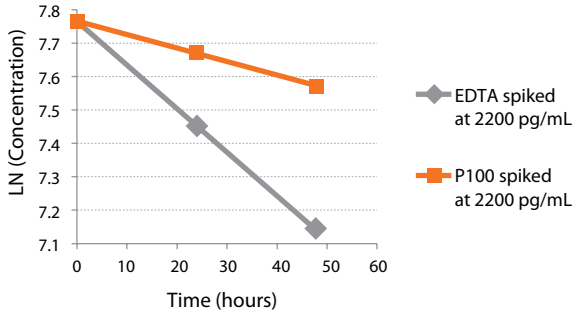
Increased stability of a known blood disease biomarker B-type natriuretic peptide (BNP) for heart failure

BNP has been identified as a good prognostic marker for morbidity and mortality associated with heart failure. It has been found that BNP levels greater than 480 pg/mL had a 51%, 6-month cumulative probability of heart failure event, whereas BNP levels of less than 250 pg/mL had better prognosis with only 2.5%, 6-month cumulative prospect of a heart failure event[7]. BNP has also been found to be the single best marker for left ventricular systolic and diastolic dysfunction and left ventricular hypertrophy allowing it to be used as a biomarker for screening and prognosis of heart failure[6].

In a study that involved 14 individuals, each individual's collected blood was spiked with 100 pg/mL, 500 pg/mL or 2200 pg/mL of BNP (total 14X3 samples). A room temperature time course experiment was performed to determine BNP stability using the ADVIA Centaur immunoassay system by Siemens Healthcare[7].

Whole Blood Stability of BNP



Half-life of BNP in BD™ P100 and EDTA

BNP spiked (pg/mL)	BD™ P100 (hours)	EDTA (hours)
100	533	64
500	301	58
2200	173	54

Number of research subjects showing more than 20% loss at 24 (left) and 48 (right) hours

24 hours		
BNP spiked (pg/mL)	BD™ P100	EDTA
100	1/14	8/14
500	2/14	7/14
2200	0/14	10/14
Total	3/42	25/42

48 hours		
BNP spiked (pg/mL)	BD™ P100	EDTA
100	2/14	14/14
500	1/14	13/14
2200	4/14	14/14
Total	7/42	41/42

Note: *Due to assay to assay variability, variation in individuals, different spiking conditions and for calculation purpose, a cut-off threshold value of 20% loss compared to time 0 was used.*

The above results showed that BNP had a significantly longer half-life in BD™ P100 Tubes compared to EDTA Tubes. At both 24 and 48 hours smaller percent loss of spiked BNP in BD™ P100 Tubes at both 24 and 48 hours suggest better stability of BNP in these Tubes. This study also showed a wide variation in BNP stability between study subjects, but this was mitigated using BD™ P100 Tubes[8]

References:

6. Cardarelli R. et al, B-type Natriuretic Peptide: B-type Natriuretic Peptide: A Review of Its Diagnostic, Prognostic, and Therapeutic Monitoring Value in Heart Failure for Primary Care Physicians, JABFP 2003, 16(4): 327-333
7. Wright G. A. et al, Natriuretic peptides as a prognostic marker and therapeutic target in heart failure, Heart 2006, 92:149-151
8. Enhanced measurement of B-type Natriuretic Peptide through the use of BD™ P100 Blood Collection Tubes containing Protease Inhibitors; Priyanka Apte; Eva A.Cronenberger, Rose Ann Juszcyk, and David Craft - BD Perter, Leaders in Biobanking 2012, Chapel Hill NC.

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Specifications and ordering information

BD™ P100 Blood Collection Tube Specifications

Total Blood Draw Volume: 8.5mL

Plasma Yield: ~3.5mL

Anticoagulant (Spray Coated): K_2EDTA

Additive (Lyophilised): Proprietary Protein Stabilisers

Storage prior to blood collection: 4˚C to 25˚C

Sterilisation: Sterility Assurance Level (SAL) 10^{-6}

Shelf life: 1 year from date of manufacture

Référence	Désignation	Condition-nement	Taille du tube	Volume de l'échantillon	Anticoagulant	Rendement en plasma
BD366448	Tubes BD P100 Blood pour prélèvement et préservation in vitro des protéines plasmatiques.	24 Tubes (4 x 6 tubes)	16 x 100 mm	8,5 ml	EDTA	env. 3,5 ml

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