PALL Life Sciences

Vivid™ Plasma Separation Membrane

Optimized, highly efficient membrane for one-step plasma separation from whole blood without the use of centrifugation.

- Achieve high quality plasma in less than two minutes with ≥ 80% plasma yield.
- Hemolysis levels significantly lower than glass fiber media generated plasma.
- Low, non-specific binding of common diagnostic biomarkers and target analytes.
- Efficient removal of the cellular components of whole blood, including red cells, white cells, and platelets.
- Compatible with POC and POU diagnostic platforms such as lateral flow test strips and microfluidics.

Specifications

Membrane
Asymmetric polysulfone

Thickness
12.99 +/- 0.79 mils (330 +/- 20 µm)

Plasma Separation Time
≤ 2 minutes

Minimum Plasma Recovery (%)
GF: ≥ 60%
GX: ≥ 60%
GR: ≥ 80%

Note: The separation time and plasma recovery data was determined using EDTA collected whole blood with a typical hematocrit content of 45.6%.

Performance

The patented Vivid Plasma Separation membrane is specifically engineered and optimized for the generation of high quality plasma for use in downstream diagnostic assays. Constructed of asymmetric polysulfone, the membrane efficiently captures the cellular components of whole blood resulting in plasma that contains minimal cellular contaminants (Table 1). The asymmetric structure of the membrane gently captures the cellular components without lysis which is a contrast from glass fiber media that often shears and lyses cells leading to contamination of the plasma (Figure 1).

Table 1
Vivid Plasma Separation Membrane Quality

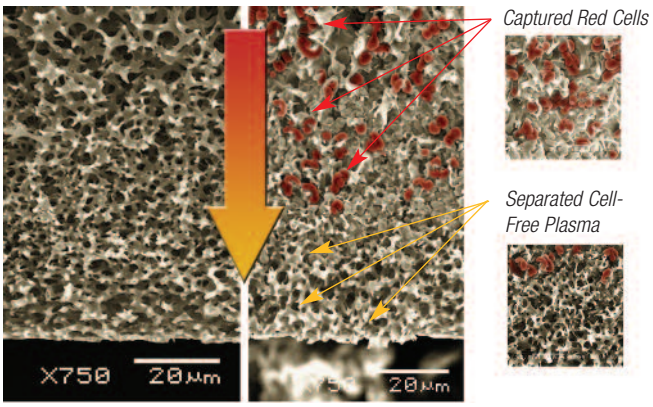
Sample	WBC (K/µL)	RBC (M/µL)	PLT (K/µL)	Hemoglobin (Hb) (g/dL)
Whole Blood	7.3	5.2	338	14.8
Centrifuged Control Plasma	0.1	0.0	7-12	0.1
Vivid Plasma Separation GF Filtered Plasma	0.0	0.0	0.0	0.1
Vivid Plasma Separation GR Filtered Plasma	0.0	0.0	0.0	0.1

Red blood cell (RBC), white blood cell (WBC), and platelet (PLT) cell concentrations, as well as red blood cell hemolysis (Hb), was measured in whole blood and centrifuged plasma samples and then compared to plasma separated with two grades of the Vivid Plasma Separation membrane. The Vivid Plasma Separation membrane effectively removes the cellular components of whole blood with low levels of hemolysis.

Performance *(continued)*

Figure 1

High Quality Plasma Generation Using Vivid Plasma Separation Membrane



The membrane can be incorporated into both lateral flow and microfluidic devices. Optimal performance can be achieved using the following guidelines.

Orientation – The asymmetric structure of the membrane results in a sidedness to the material. When removing the sheet of membrane from its package, the shiny side will be facing up towards the label. Prior to using the membrane, ensure that you have identified the shiny side from the dull side. To achieve the desired plasma separation, you must apply whole blood to the dull side of the membrane. Applying whole blood to the shiny side of the membrane will not result in the desired plasma generation.

Blood Volume Rule – For optimal plasma yield and separation time, care needs to be taken to ensure that the membrane is not overloaded with whole blood. The blood volume capacity of the membrane is directly related to its void volume (e.g., the space within the solid phase of the membrane that is occupied by a liquid sample). The calculated void volume of Vivid Plasma Separation membrane is ~20 ± 1 µL/cm^2. The sample blood volume capacity of Vivid Plasma Separation membrane is defined as the amount of whole blood per cm^2 of membrane that is rapidly and consistently separated (< 2 min.) by the membrane with low levels of hemolysis. With knowledge of the void volume within membrane and the grade characteristics, Pall recommends the following blood volume capacities that generate rapid and consistent plasma separation with low hemolysis (Table 2).

Table 2

Blood Volume Recomendations

Membrane Grade	Recommended Whole Blood Sample Volume (µL/cm^2)	Membrane Void Volume
GF	20 µL	1X
GX	20-30 µL	1-1.5X
GR	40-50 µL	2-2.5X

Grade Selection – There are three grades of Vivid Plasma Separation membrane, each optimized for various usage conditions.

- **GF** – Small blood volume applications, such as finger sticks in formats like microfluidic and lateral flow POC devices. No post-treatment so material may exhibit higher hemolysis levels than other grades.
- **GX** – Small blood volume applications, such as finger sticks in formats like microfluidic and lateral flow POC devices. Also compatible with electrochemical analyte detection. Post-treatment helps to minimize hemolysis.
- **GR** – Larger blood volume applications, such as lateral flow immunochromatographic devices. Post-treatment facilitates larger blood volumes with lower hemolysis.

Device Construction – Ensuring proper contact of the membrane with the receiving medium is critically important. Plasma does not freely flow from the bottom of the membrane and requires uniform contact with a receiving matrix for the plasma to be delivered to the test strip or device. Plasma transfers from the bottom of Vivid Plasma Separation membrane to the receiving medium by capillary forces of the receiving medium. The underlying material or structure must have enough capillary force to wick the plasma from the membrane for efficient delivery of plasma. Lamination or sealing of the two materials should be performed to ensure that there is intimate contact between them, but that the asymmetric structure of the membrane is not compressed or disturbed.

When sealing the membrane into your device, consider the following:

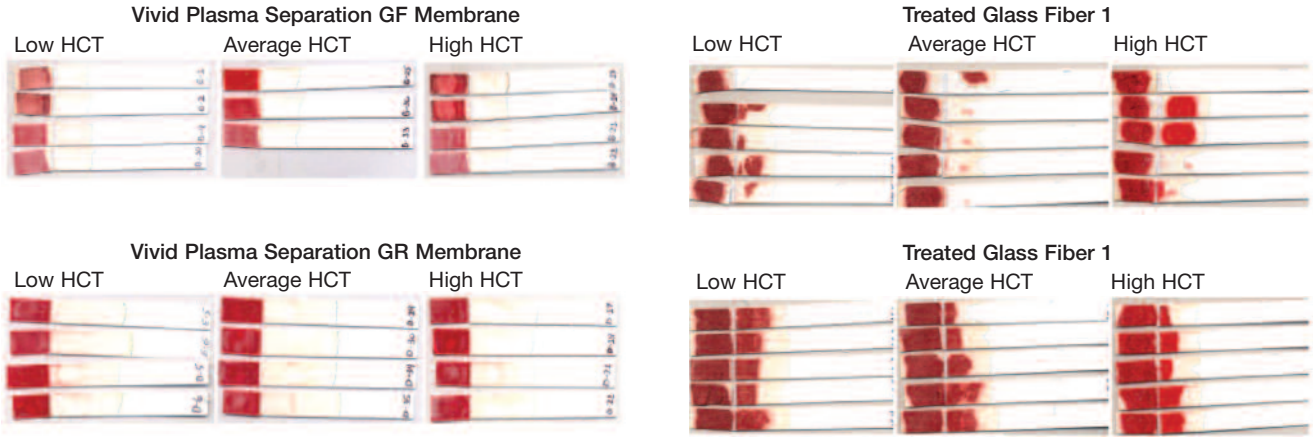
- Ensure that there are no air gaps/bubbles between the membrane and the receiving matrix.
- Apply pressure around the membrane pad to prevent blood spilling at the edges of the blood separation pad, but do not apply so much pressure that it prevents the flow of the plasma along the receiving material. The sealing pressure should be optimized for the device configuration.

- The hydrophobic/hydrophilic characteristics of the membrane and the receiving materials should be compatible for optimal plasma collection.
- Chemical treatments applied to the receiving material should not cause hemolysis of the red cells in the upstream blood samples resulting in hemolysis in the receiving plasma.

High Plasma Yield – The Vivid Plasma Separation membrane provides high plasma yields lowering the amount of starting whole blood needed. For point-of-care (POC) and point-of-use (POU) diagnostic applications, this is advantageous as whole blood volumes can be minimized resulting in smaller amounts of blood needed from patients or animals. The GR grade membrane provides ≥ 80% yield of the theoretical plasma available while our GF and GX variants provide ≥ 60% yield. Comparatively, glass fiber yields are only 30-50% resulting in much larger starting volumes of whole blood required to achieve the same amount of plasma (see Figures 2 and 3).

Figure 2

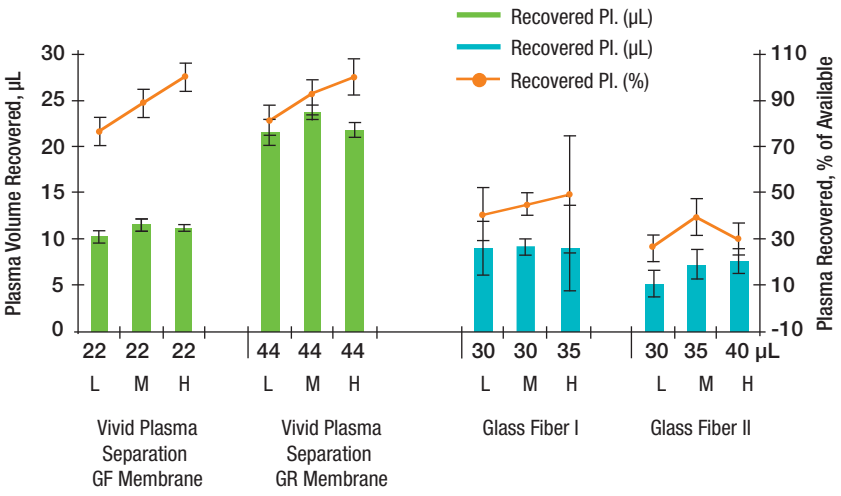
Generate High Quality Plasma with Vivid Plasma Separation Membrane vs. Glass Fiber Media



Test strips were composed of Vivid Plasma Separation membrane over nitrocellulose membrane. Test strips were challenged with samples of whole blood that was applied to the media in volumes optimal for the grades of Vivid Plasma Separation membrane or as recommended by a manufacturer for glass fiber grades. Vivid Plasma Separation membrane test strips are presented in the left two panels, glass fiber test strips are presented in the right two panels. Notice that blood separation pads were flipped aside of the receiving media after blood separation completion in order to show the quality of recovered plasma directly under the pad. Blood samples of low, 37% (left columns); medium, 42% (middle columns); and high, 48% HTC (right columns) were used for the test.

Figure 3

Achieve Greater Plasma Yields with Vivid Plasma Separation Membrane vs. Glass Fiber Media



The use of Vivid Plasma Separation membrane allows for greater efficiencies in plasma separation, yield, and quality at all hematocrit levels compared to glass fiber media. In addition, larger volumes of plasma from smaller volumes of whole blood were recovered by Vivid Plasma Separation membrane compared to glass fiber media.

The numbers below the bars on the chart represent the amount of whole blood applied to the membrane in µL per square centimeter. Beneath the bars, the letters L, M, and H represent the hematocrit levels of the whole blood applied. The low (L) hematocrit level is 37%, medium (M) is 42%, and high (H) is 48%.

Low Analyte Binding – A common concern when using polymeric separation materials is the non-specific binding of target analytes, proteins, and biomarkers to the material. It is well known that some key analytes and biomarkers (e.g., cholesterol, ∅9 THC) have a tendency to bind to membranes used in diagnostic assays which hinders the accuracy and sensitivity of the assay. The Vivid Plasma Separation membrane exhibits low, non-specific binding of key diagnostic biomarkers such as Troponin I. A comparison of plasma generated with the Vivid Plasma Separation membrane to centrifuged control plasma was conducted to assess the detection of Tropinin I in plasma generated by the Vivid Plasma Separation membrane.

Model: Detection of Human Cardiac Troponin I in plasma samples generated by Vivid Plasma Separation membrane as well as centrifugation.

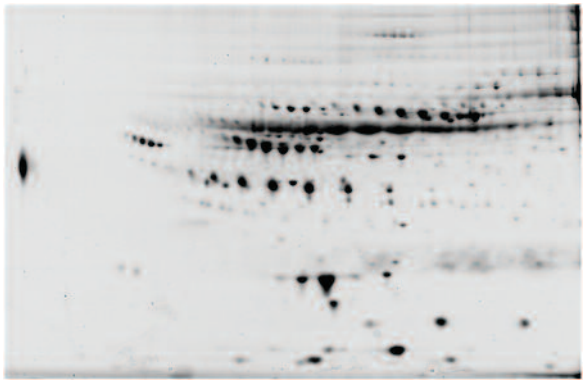
- Whole, fresh EDTA blood was spiked with 1 ng/mL of Troponin I.
- Plasma samples were generated by gravity filtration of spiked blood through Vivid Plasma Separation membrane. Two grades of the media, GF and GR, were used in the study.
- Control plasma was obtained by centrifuging 200 µL of whole blood spiked with Troponin I at 1,000 x g for 5 minutes.
- Troponin I concentration in obtained plasma was measured by ELISA. Additionally, 2D gel analysis was performed to compare protein profiles of the plasma. The 2D gel images are shown in Figure 4–A, B, and C.

The 2DE and HPLC profiles of the two plasmas were evaluated to assess the tendency of the membrane to bind target biomolecules. The findings showed that the protein profiles of the Vivid Plasma Separation membrane generated plasma were statistically identical to the profiles generated by centrifugation (Figures 5–A, B, and C). Therefore, the use of the Vivid Plasma Separation membrane in diagnostic assays minimizes the concern over target analyte binding for the Troponin I biomarker (Figure 6).

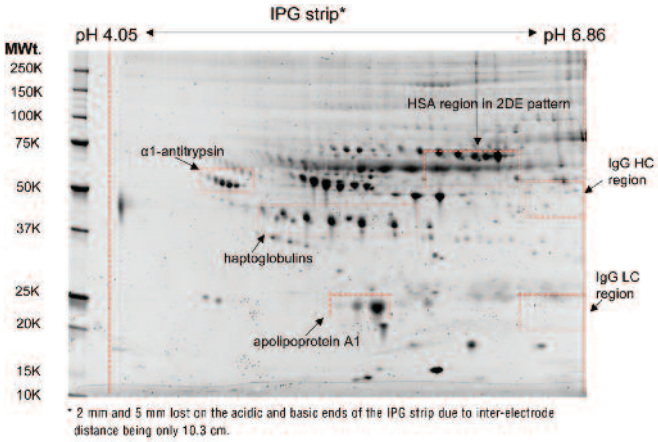
Figure 4

Low, Non-Specific Binding of Target Analytes with Vivid Plasma Separation Membrane Generated Plasma

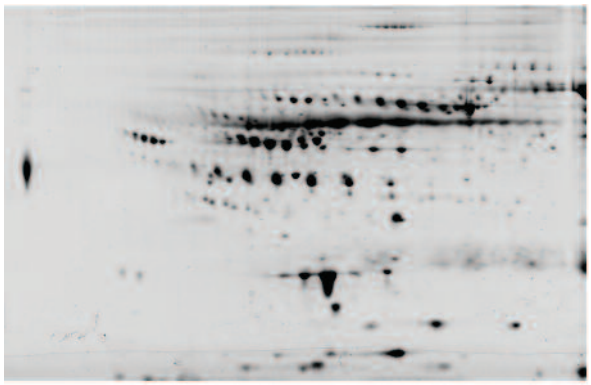
A **Vivid Plasma Separation GF Membrane Generated Plasma**



B **Centrifuged Control Plasma**



C **Vivid Plasma Separation GR Membrane Generated Plasma**



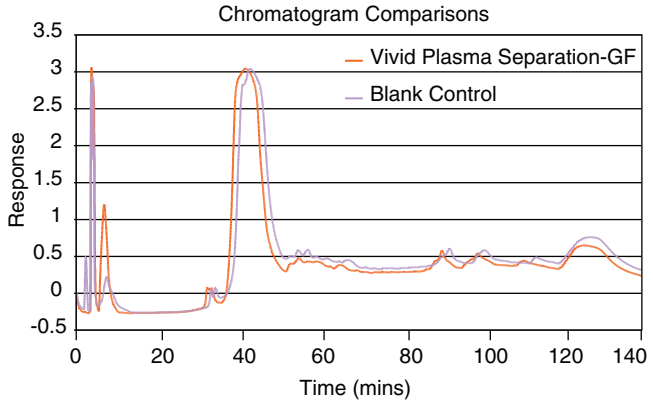
Picture A-C: 2D gel electrophoresis protein profiles of control centrifuged sample (gel B) and Vivid Plasma Separation membrane generated plasma (gel A and C).

A 2DE protocol of very high resolution of the acidic pI – medium molecular weight region where most of the known cardiac biomarkers are located, employed a first dimension with pH 4-7 NL IPG strips followed by second dimension separation on 10.5-14% SDS PAGE.

Figure 5

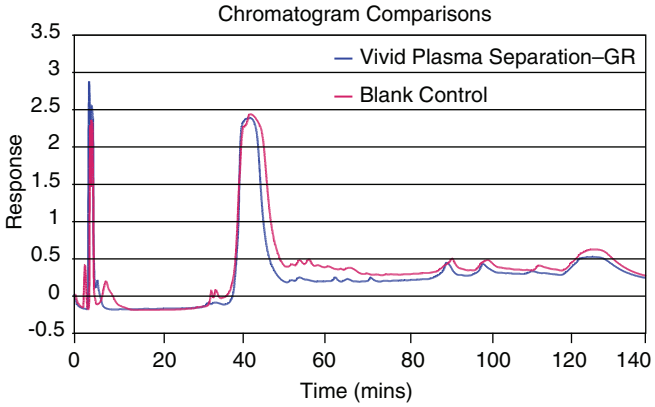
Equivalent HPLC Profiles Using Vivid Plasma Separation Membrane

A Vivid Plasma Separation GF Membrane vs. Control



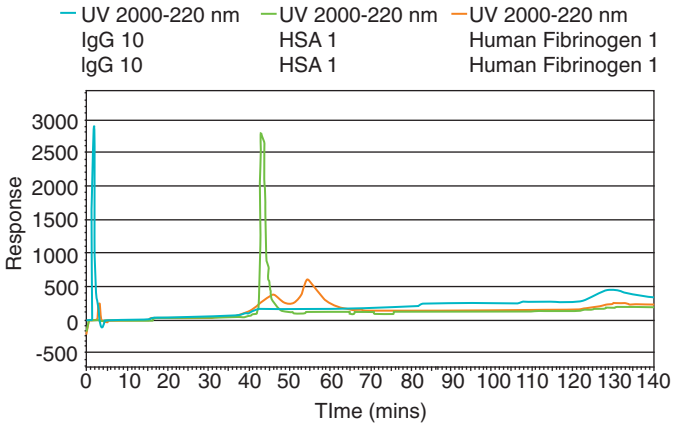
HPLC profiles of plasma filtered through Vivid Plasma Separation GF membrane (red line) vs. centrifuged plasma (purple line). Filtered and centrifuged plasma resulted in very similar profiles.

B Vivid Plasma Separation GR Membrane vs. Control



HPLC profiles of plasma filtered through Vivid Plasma Separation GR membrane (blue line) vs. centrifuged plasma (red line). **Note:** *There was a small decrease in protein concentrations in areas of HSA and Fibrinogen observed in plasma filtered through Vivid Plasma Separation GR membrane.*

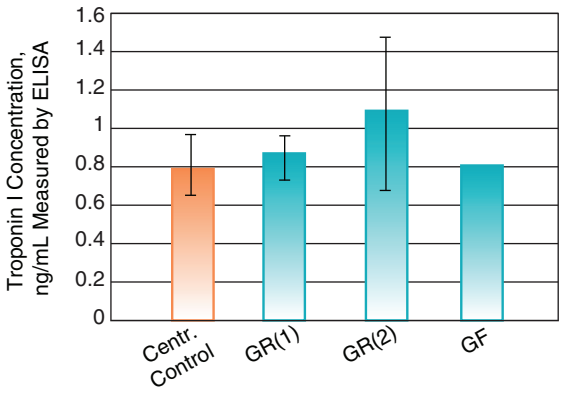
C HPLC Profiles of Abundant Proteins



HPLC profiles of abundant human plasma proteins, IgG, Human Serum Albumin (HSA), and Fibrinogen that were purchased from Sigma.

Figure 6

Concentration of Cardiac Biomarker Maintained Using Vivid Plasma Separation Membrane Filtered Plasma



Troponin I concentration was measured in plasma samples filtered through Vivid Plasma Separation membrane (teal columns) vs. centrifuged plasma (orange column). All plasma samples were generated from the same sample of fresh EDTA blood spiked with Troponin I (1 ng/mL). Concentrations of Troponin I are equivalent in centrifuged and filtered plasma samples indicating that the Vivid Plasma Separation membrane exhibits low levels of non-specific binding of key cardiac biomarkers.

Ordering Information

Part Number	Description	Pkg
T9EXPPA0200S00A	Vivid Plasma Separation GF membrane, 8" x 11" sheet	1/pkg
T9EXPPA0200S00X	Vivid Plasma Separation GX membrane, 8" x 11" sheet	1/pkg
T9EXPPA0200S00R	Vivid Plasma Separation GR membrane, 8" x 11" sheet	1/pkg

Complimentary Products

- **Vivid 170 Nitrocellulose Membrane** exhibits tightly controlled thickness and wicking rates to help facilitate assay sensitivity and reproducibility in lateral flow assays.
- **White Blood Cell Isolation Medium** isolates leukocytes from whole blood samples. The nucleic acid content can be extracted for further analysis in molecular detection applications.
- **Conjugate Pads** are available in a variety of thicknesses and materials of construction to optimize assay performance.
- **Absorbent Pads** constructed of pure cellulose fibers are available in a variety of absorbencies depending on sample type and volume.
- **AcroPrep™ Filter Plates** offer superior performance for high throughput sample preparation procedures.

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