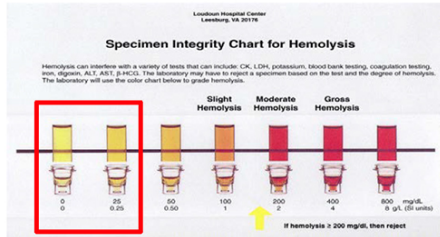


# ProteinGard™ Application Note

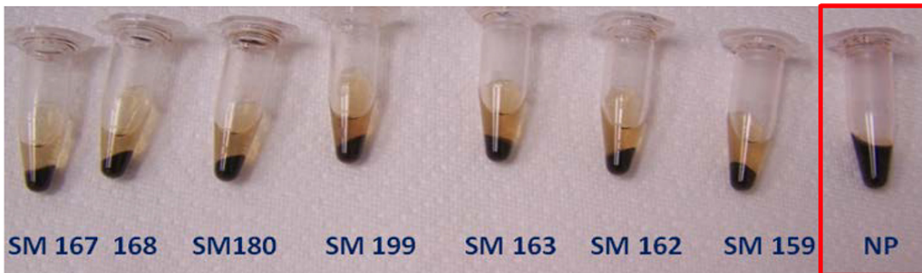
Biomatrix's ProteinGard technology allows stabilization of native proteins in aqueous environments, such as human plasma, at room temperature. The technology has been validated with numerous proteins, including cytokines that are known to be intrinsically unstable and diagnostically relevant, e.g., IL-6, TNF, IL-10 and IL-1 $\beta$ . Additionally, ProteinGard stabilizers have been shown to inhibit hemolysis allowing accurate quantitation of proteins in whole blood samples.

## Controlling Hemolysis at Ambient Temperature



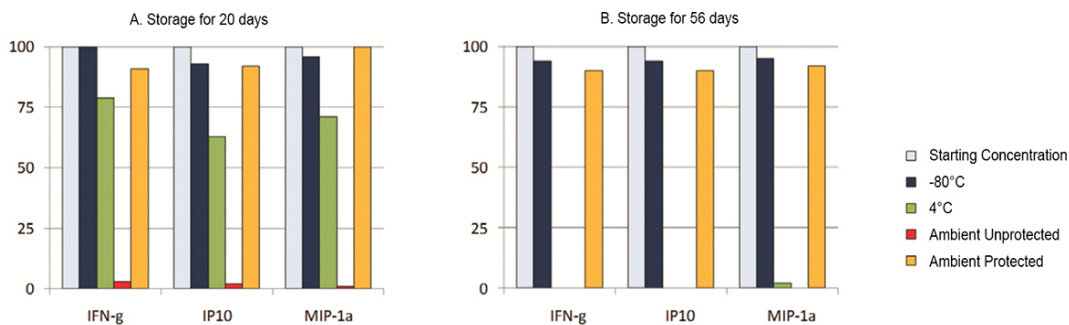
Selection criteria are selected to create plasma with <0.5g/l (SI units)

EDTA Collection tube



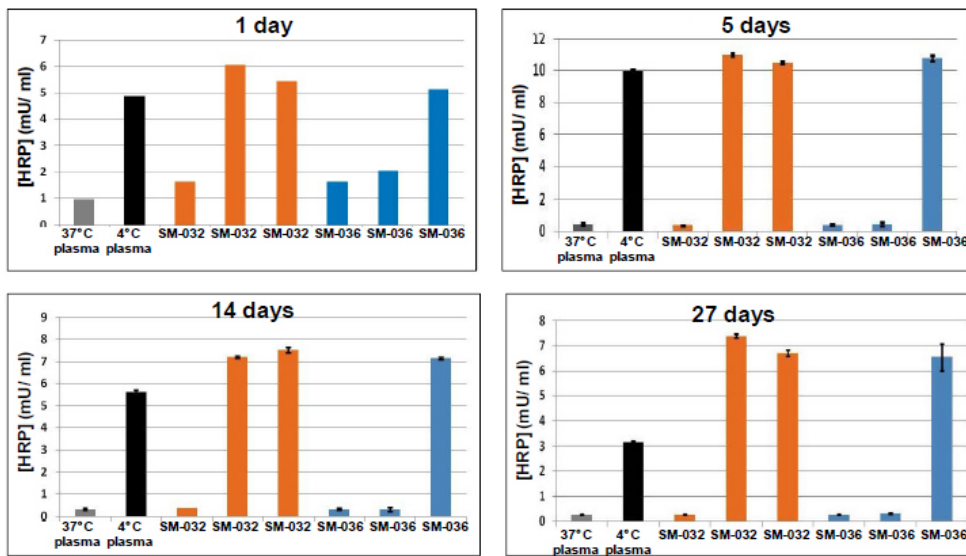
**Figure 1: Examples of protein stabilization formulations impact on cell lysis.** Blood/formulation mixtures of 1:1 (v/v) were incubated for 23 days at room temperature. EDTA was used as a non-stabilized negative control (NP). Hemolysis in blood samples was assessed visually using the BD Specimen Integrity Chart for Hemolysis above.

## Native Protein Stabilization in Plasma



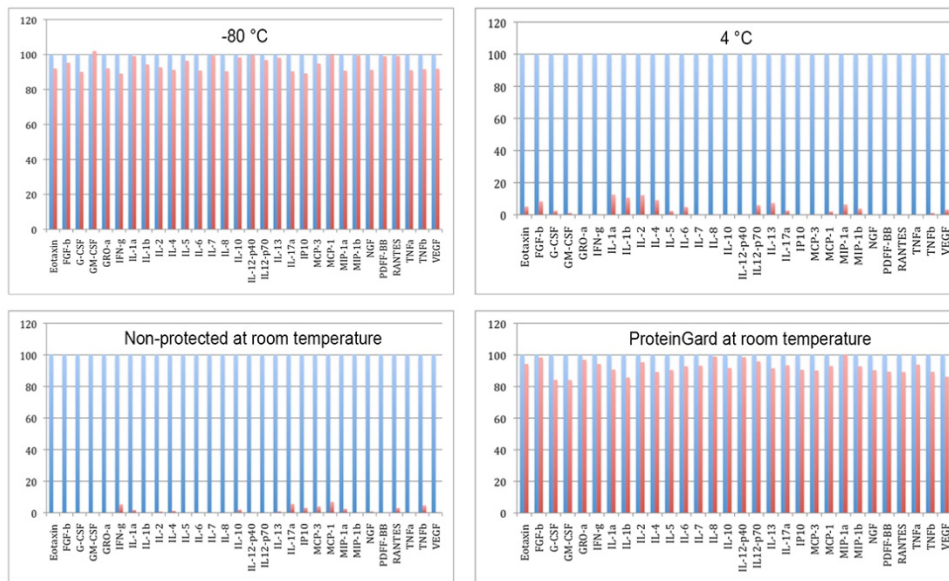
**Figure 2: Cytokine stability during ambient temperature storage in human plasma.** Human plasma was spiked with 10 ng protein and stored at ambient temperature in the absence (red bars) or presence (orange bars) of Biomatrix formulations. Control specimens without stabilizer were stored at 4 °C (green bars) or -80 °C (dark blue bars). Cytokine levels were measured after 20 days (left graph) and 56 days (right graph) of storage using the Luminex system with a Panomix human cytokine array and plotted relative to cytokine levels at time 0 (light blue bars).

## Native Protein Stabilization in Plasma at 37°C



**Figure 3: Horseradish peroxidase stabilization in plasma at 37°C.** HRP activity remaining in plasma spike-in specimens stored at 37°C was measured at indicated time points. Plasma specimens stored without added stabilizer at 4°C or 37°C are shown in black and gray, respectively. Specimens incubated at 37°C with Biomatrix formulations are shown in orange and blue.

## Integrity of Plasma Cytokines Stored for 56 Days at Room Temperature



**Figure 4: Biomatrix biostability compounds protect cytokines in plasma during room temperature storage.** Panomics human cytokine array of plasma was spiked with 10 ng/standard after 35 day storage at -80°C (% cytokine). Blue bars represent t=0 control, red bars represent t=35 days. Samples were diluted 100-fold prior to cytokine quantification. Neat plasma with spiked standards was used at equivalent dilution as a t=0 control; its concentration was normalized to 100% to allow cross comparison on an equivalent scale. The experiment was performed for plasma controls stored at -80°C, 4°C, and at room temperature as a non-stabilized sample (20 days), or stabilized at room temperature using Biomatrix's liquid stabilization technology (56 days). During storage at room temperature, the stabilized plasma does not show any sign of degradation, and the level of stability is equivalent to material stored at -80°C.