

NuGel-HemogloBind[™]

Hemoglobin Removal with NuGel™ Dry-Bead Format

- Has a high degree of specificity for hemoglobin binding up to 10 mg/ml
- Species agnostic; including human, mouse, rat, sheep, bovine, goat, etc
- Removes hemoglobin from clarified organ/tissue lysates.
- Hemoglobin removal from red blood cell lysate for proteomics and biomarker drug discovery
- The flow through fractions (hemoglobin depleted) retain their enzymatic and biological activity
- Compatible with LC-MS, activity-based profiling and proteomic investigations
- Validated in the automation compatible high-throughput DPX Technologies XTR tip format

NuGel[™] Hemoglobind[™] is a modified version of suspension HemogloBind[™], engineered for increased stability and improved installation for high-throughput formats. It is based on NuGel[™] silica (50 microns in size, 1000Å) covalently bound to elastomeric polyelectrolytes. Comparable to selection properties of suspension HemogloBind[™], at recommended quantities, >95% of hemoglobin can be removed with very high selectivity.





LC-MS data from NuGel[™] HemogloBind[™] installed within the high-throughput DPX Technologies XTR tip format

Representative Enriched Proteins after NuGel HemogloBind (Uniprot ID)	Description	Sp. Cts control	Sp. Cts after	LC-MS Proteomic Analysis of Serum		Whole Blood	After NuGel™ HemogloBind™
sp P01023 A2MG_HUMAN	Alpha-2-macroglobulin	376	896	(single 2 nr		Lysale Control	Hemoglobinu
sp P04114 APOB_HUMAN	Apolipoprotein B-100	280	660	gradie	ent) DDA		
sp P02751-1 FINC_HUMAN	Fibronectin	120	248				
sp P43652 AFAM_HUMAN	Afamin	23	54	Total Spe	ctral Counts		
sp P11277 SPTB1_HUMAN	Spectrin beta chain	32	130	15		14532	19054
sp P02549 SPTA1_HUMAN	Spectrin alpha chain	39	163	(2)	J. C(S)		
sp P04003 C4BPA_HUMAN	C4b-binding protein alpha chain	15	50				
sp P08697 A2AP_HUMAN	Alpha-2-antiplasmin	19	48	Total Pro	tein IDs (≥2		
sp Q06033 ITIH3_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H3	14	43	Sp. Cts)		306	420
sp P02730 B3AT_HUMAN	Band 3 anion transport protein	42	110				
sp P05543 THBG_HUMAN	Thyroxine-binding globulin	7	27				
sp P09871 C1S_HUMAN	Complement C1s subcomponent	9	34	# of Uni	nue Protein		
sp 075882 ATRN_HUMAN	Attractin	4	18			27	134
sp P16157 ANK1_HUMAN	Ankyrin-1	18	73	IDs (≥	2 Sp. Cts)		
sp P04406 G3P_HUMAN	Glyceraldehyde-3-phosphate dehydrogenase	28	61				
sp P02750 A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein	4	13	<u> </u>	- nui ala a d		
sp P62258 1433E_HUMAN	14-3-3 protein epsilon	11	51	# of Enriched Proteins ≥3X			47
sp P20742 PZP_HUMAN	Pregnancy zone protein	7	36				
tr G3V1D3 G3V1D3 HUMAN	Dipeptidyl peptidase 3	9	42				
tr C9JIF9 C9JIF9 HUMAN	Acyl-peptide hydrolase	11	38	# of L	Depleted		
sp P15169 CBPN_HUMAN	Carboxypeptidase N catalytic chain	2	14	Proteins (other than Hemoglobin		<i>c</i>	
sp P05160 F13B_HUMAN	Coagulation factor XIII B chain	1	13			b	
sp P16452 EPB42_HUMAN	Protein 4.2	5	38	cubu			
sp Q13630 FCL HUMAN	GDP-L-fucose synthase	8	33	Subu	1115) 251		
sp P09960 LKHA4 HUMAN	Leukotriene A-4 hydrolase	9	28	Homoglobin Doplation		n	
sp P22314-2 UBA1 HUMAN	Ubiquitin-like modifier-activating enzyme 1	8	28	40. Hemoglobin Depiction			
sp P07900 HS90A_HUMAN	Heat shock protein HSP 90-alpha	3	28	OD414 (Avg 2 readings)		gs)	
sp P12955 PEPD_HUMAN	Xaa-Pro dipeptidase	3	23	50.			
sp P23142 FBLN1 HUMAN	Fibulin-1	4	19	20.			
sp P25786 PSA1_HUMAN	Proteasome subunit alpha type-1	2	25	10			
sp[P31946]1433B_HUMAN	14-3-3 protein beta/alpha	4	18	10.			
splP459741UBP5_HUMAN	Ubiguitin carboxyl-terminal hydrolase 5	2	20	0			
splo6XON6/PNCB_HUMAN	Nicotinate phosphoribosyltransferase	3	17	0.			
splP092111GSTP1_HUMAN	Glutathione S-transferase P	- 5	15		Whole Blo	od 5 Cycles	10 Cycles
sp1P619701NTF2_HUMAN	Nuclear transport factor 2	4	15		Lvsate	Binding	Binding
trifetix2/fetix2_human	Glyoxalase domain-containing protein 4	4	15		,	- 0	. 0
splP07996ITSP1 HUMAN	Thrombospondin-1	nd	19				
splP280701PSB4_HUMAN	Proteasome subunit beta type-4	4	15	Fr	om a poste	r report describi	ng NuGel™
	Consulation factor XIII A chain	1	16	Homoglo Pind [™] installed within the high			
sp11004881113A_110101A0		T	10				
sp P21980 TGM2_HUMAN	glutamyltransferase 2	1	14	throughput DPX Technologies XTR tip format. For			
sp Q14974 IMB1 HUMAN	Importin subunit beta-1	nd	14	full poster report, go to:			
sp P53396 ACLY_HUMAN	ATP-citrate synthase	1	13	https:/		acheunnartereu	n com hy hycofile
sp P06132 DCUP_HUMAN	, Uroporphyrinogen decarboxylase	1	13	nttps://www.biotecnsupportgroup.com/v/vspfile			
tr B3KQV6 B3KQV6_HUMAN	Serine/threonine-protein phosphatase 2A	nd	10	s/templates/257/pdf/ASMSBSGDPXPoster.pdf			
1	,		I	l			



Product	Size	Total Sample Processed	Product Code	
NuGel-HemogloBind™	25 preps	500µl of blood or 5 ml of Hemolyzed Serum or Plasma	NP-HO-T25	
NuGel-HemogloBind™	50 preps	1 ml of blood or 10 ml of Hemolyzed Serum or Plasma	NP-HO-T50	
NuGel-HemogloBind™	5 preps	100µl of blood or 1 ml of Hemolyzed Serum or Plasma	Included in HemoTrial [™] kit (HTK-05)	
NuGel-HemogloBind™5 preps100µl o Hemolyze		100µl of blood or 1 ml of Hemolyzed Serum or Plasma	Included in HemogloBind™ Trial kit (HB145K)	

Items Required	5 Prep	25 Prep	50 Prep	Reagent
NuGel-HemogloBind™ beads	0.25 gram	1.25 grams	2.5 gram	Supplied
Hemoglobin Binding Buffer (HB) 0.05M K ₂ HPO ₄ pH 6.5	3 ml	15 ml	30 ml	Supplied
Spin-filter / tube assemblies	5	25	50	Supplied

Additional Spin-Filters (low protein binding, 0.45 μ m filter element) can be purchased separately, please inquire.

Guidelines for determining the quantity of NuGel[™] HemogloBind[™] to sample. Use this chart to estimate the amount of Hemoglobin in samples containing mostly Hemoglobin (i.e., lysed erythrocytes) or mostly serum/plasma proteins (i.e., hemolyzed serum). Adjust bead amounts as necessary to optimize for investigative goals.





Notes: Hemoglobin concentrations in hemolyzed serum/plasma vary greatly. Protocols are intended as guidelines, but it is recommended to try different amounts of beads relative to sample for optimal results.

The protocol can be scaled up or down proportionally to adjust for different sample volumes. The bead amount can be adjusted to accommodate more or less Hemoglobin removal.

For best results – the lysate should be clear and free of colloidal material. We recommend first filtering through a 0.45 μ m syringe-type filter before beginning the prep. Depending upon the quality of the sample, centrifugation times can be adjusted to increase g's or time, sufficient to process the sample through the beads.

PROTOCOL – To Treat Whole Blood (10-20 µl) Sample

- BEAD CONDITIONING. Weigh out 50 mg of NuGel-HemogloBind[™] beads in a Spin-filter tube. Add 200 µl of Hemoglobin Binding Buffer (HB) to the beads. Vortex or mix well for 2 minutes at room temperature. Centrifuge for 2 minutes at 1,000 g's. Discard the filtrate.
- 2. **SAMPLE CONDITIONING.** In a separate microfuge tube, add 200-400 μl of **Hemoglobin Binding Buffer** (**HB**) and 10-20 μl of blood sample. Vortex for 3 minutes.
- 3. **SAMPLE PROCESSING.** Add sample from step 2 to conditioned beads from step 1.
- 4. Vortex or mix well for 10 minutes at room temperature followed by centrifugation for 4 minutes at 5,000 g's.
- 5. Collect the filtrate which contains the hemoglobin depleted sample suitable for further analysis; the retained material contains the hemoglobin removed.

PROTOCOL – To Treat Hemolyzed Serum/Plasma (200 µl) Sample

- 1. **BEAD CONDITIONING.** Weigh out 50 mg of **NuGel-HemogloBind**[™] beads in a Spin-filter. Add 400 µl Hemoglobin Binding Buffer. Vortex or mix well for 2 minutes at room temperature. Centrifuge for 2 minutes at 1,000 g's. Discard the filtrate.
- 2. **SAMPLE PROCESSING.** Add 200 µl Hemolyzed sample to the conditioned beads.
- 3. Vortex or mix well for 10 minutes at room temperature followed by centrifugation for 4 minutes at 5,000 g's.
- 4. Collect the filtrate which contains the hemoglobin depleted sample suitable for further analysis; the retained material contains the hemoglobin removed.

Desorption of Bound Hemoglobin

For purification and/or analysis of hemoglobin, 100 mM Tris-Borate, pH 9, will facilitate desorption of hemoglobin bound to HemogloBind[™].

Hemoglobin Variants

Hemoglobin variants, as in thalassemia and glycosylated-hemoglobin, can also be analyzed with **HemoVoid™ - Hemoglobin Variant Enrichment**, see link

https://www.biotechsupportgroup.com/HemoVoid-Hemoglobin-Variant-Enrichment-From-Blood-p/hbv.htm



Validated in the high-throughput XTR tip format

The XTR tip format improves ease of use and scalability to process multiple samples in parallel, utilizing 96-well plates and automated liquid handlers. INTip[™] SPE formats have been proven to be compatible with most automation platforms, i.e., Integra, Hamilton, etc. The **NuGeI[™] HemogloBind[™]** beads are loosely contained inside the XTR tips for a dispersive functionality that maximizes depletion efficacy.

A poster report is downloadable at: https://www.biotechsupportgroup.com/v/vspfiles/templates/257/ pdf/ASMSBSGDPXPoster.pdf

TAT	KDPX INTip™ SPE
	Barrier
	Loose Sorbent Albedre ^{My} NoGe ^{My} HenogleBind ^{My}

Please inquire for price and availability.

Selection of NuGel[™]HemogloBind [™] and related HemogloBind[™] References

Leitner, Dominique F., et al. "<u>Metabolomic, Proteomic, and Transcriptomic Changes in Adults with Epilepsy on Modified Atkins</u> <u>Diet</u>." *Epilepsia* (2023). For Plasma Metabolomics, the article states "Whole blood was thawed on ice and processed to remove hemoglobin by **NuGel-HemogloBind** according to manufacturer protocol..."

Jara, Zaira Palomino, et al. "<u>Distinct Mechanisms of β-Arrestin-Biased Agonist and Blocker of AT1R in Preventing Aortic Aneurysm</u> and Associated Mortality." *Hypertension* 80.2 (2023): 385-402. The article states "Cleaned abdominal aortas were homogenized in T-PER Tissue Protein Extraction reagent.... **Abdominal aneurysm samples were treated to remove hemoglobin captured within the vessel wall. NuGel-HemogloBind ... was used** according to the manufacturer protocol."

Zhang, X., Li, S., Malik, I. *et al.* Reprogramming tumour-associated macrophages to outcompete cancer cells. *Nature* (2023). <u>https://doi.org/10.1038/s41586-023-06256-5</u> To measure the amino acid content by LC-MS from tumor interstitial fluid, the article states "Samples were brought through a NuGel-HemogloBind prep prior to extraction to remedy the levels of haemolysis present.

Chen, Yaozhen, et al. "Red blood cells undergo lytic programmed cell death involving NLRP3." *Cell* (2025). The article states "**Hemoglobin was depleted by adding 250 µl of HemogloBind suspension** to each lysate. ...the hemoglobindepleted supernatant...was then subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis". Gene Ontology (GO) analysis revealed significant enrichment of pathways associated with cytoskeleton organization and actin filament assembly dynamics.

de Boni, Laura, et al. "Aggregation-resistant alpha-synuclein tetramers are reduced in the blood of Parkinson's patients." *EMBO Molecular Medicine* (2024): 1-18. Synucleinopathies such as Parkinson's disease (PD) are defined by the accumulation and aggregation of the α-synuclein protein in neurons, glia and other tissues. In this study, an in vitro-cross-linking protocol for human EDTA-whole blood was used to determine the relative levels of disordered and higher-ordered multimeric forms of cytosolic αsynuclein in blood. The protocol incorporated **HemogloBind[™] to remove interference from Hemoglobin**.

For a full list of Hemoglobin Removal references, visit: https://www.biotechsupportgroup.com/References-s/138.htm#hemoglobin-depletion

CONTACT US

We welcome your questions, comments and concerns regarding our products.Call732-274-2866, Monday – Friday 9am-5pm EST.Emailsales@biotechsupportgroup.com