

Supporting Information:

Relative yields of the llama IgG subclasses after protein G and protein A affinity chromatography

IgG subclass	Yield [mg/ml serum]	[% of total cIgG]
Total cIgG	26,3 ± 2,5	100
IgG3	3,9 ± 1,1	15,1 ± 4,2
IgG1	15,6 ± 2,1	59,4 ± 7,9
IgG2a	3,5 ± 0,5	13,2 ± 2,0
IgG2b	3,2 ± 0,6	12,2 ± 2,3

Immobilisation efficiency of the tested resins (1 ml resin slurry)

	CNBr-Sepharose	NHS-Sepharose	Protein G-Agarose	Hydrazid-Agarose
input_{IgG3} [µg]	1000	1000	1000	1000
flow through [µg]	168.8 ± 6.6	150.8 ± 4.3	924.8 ± 21.0	160.9 ± 7.3
bound_{IgG3} [%]	83.1 ± 0.7	84.9 ± 0.4	7.5 ± 2.1	83.9 ± 0.7

Bindung capacity of the antibody affinity columns (1 ml resin slurry)

	CNBr-Sepharose	NHS-Sepharose	Hydrazid-Agarose
input_{antigen} [µg]	50	50	50
flow through [µg]	3.97 ± 0.6	4.07 ± 0.3	2.03 ± 0.3
flow through [%]	7.94 ± 1.2	8.14 ± 0.6	4.06 ± 0.6
column recycling	~ 15 x	> 40 x	> 40 x

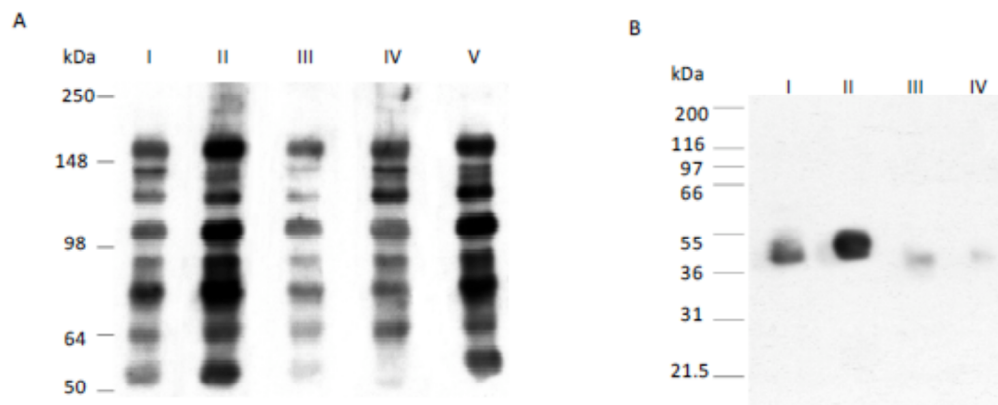


Figure: Antigen specificity of the llama IgG subtypes (I: IgG3, II: IgG1, III: IgG2a, IV: IgG2b, V: unfractionated llama serum): 4 μ g WGA-bound serum proteins per lane hybridized with 0.1 μ g/ μ l antibody solution 1b: Differential sensitivity of the secondary antibody: 0.1 μ g of each IgG subtype per lane hybridized with the secondary antibody (goat anti-llama HRP conjugated antibody)

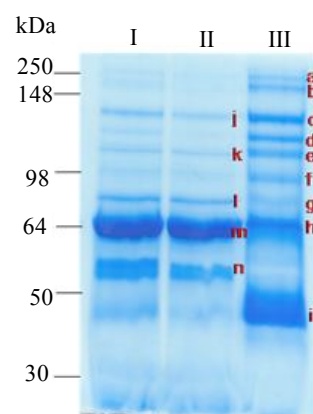


Figure: SDS-PAGE of the plasma protein fractions after WGA affinity chromatography (I: plasma II: flow through III: WGA-bound proteins)
a-n: proteins identified by tandem mass spectrometry:
a: apolipoprotein A, b: apolipoprotein B, c,f,: inter-alpha-trypsin inhibitor heavy chain,

d-f,k: α_2 -macroglobulin, g: plasma protease C1 inhibitor, h,m: albumin, h: histidin-rich glycoprotein, i: hemopexin (beta-1B-glycoprotein), n: IgG heavy chain

Table: Proteins identified by LC/MS^E in normal human plasma

(sorted according to significance scores)

rank	identified proteins
1	serum albumin
2	serotransferrin
3	complement C3
4	complement C4-A
5	complement C4-B
6	Ig gamma-1 chain C region
7	haptoglobin
8	apolipoprotein A-I
9	hemopexin (beta 1B-glycoprotein)
10	fibrinogen beta chain
11	fibrinogen gamma chain
12	fibrinogen Alpha chain
13	ceruloplasmin (ferroxidase)
14	complement factor H
15	Pregnancy-zone protein
16	Ig kappa chain C region
17	Ig gamma-2 chain C region
18	Alpha-1 acid glykoprotein 1 (orosomucoid-1)
19	vitamin D-binding protein
20	Inter-alpha-trypsin inhibitor heavy chain H4
21	Ig gamma-4 chain C region
22	Haptoglobin-related protein
23	complement factor B
24	Ig alpha-1 chain C region
25	Ig lambda chain C regions
26	plasminogen
27	Alpha-1 antichymotrypsin
28	Beta-2 glycoprotein 1 (apolipoprotein H)
29	Alpha-1 antitrypsin (alpha-1 protease inhibitor)
30	Alpha-1B glycoprotein (alpha-1B glykoprotein)
31	Ig gamma-3 chain C region
32	complement component C6
33	protein cTAGE-6
34	Ig alpha-2 chain C region
35	leukotriene A-4 hydrolase
36	Clusterin (Apolipoprotein J)
37	protein regulator cytokinese 1
38	integrin alpha-M
39	Alpha-2 HS glycoprotein (fetuin A)
40	prothrombin
41	Ig mu heavy chain

42	Ig kappa chain V-III region WOL
43	Alpha-2 macroglobulin
44	Caspase-4
45	apolipoprotein C-III
46	Ig kappa chain V-III Region SIE
47	Alpha-1 acid glykoprotein 2 (orosomucoid-2)
48	Ig Mu chain C region
49	Apolipoprotein E (Apo E)
50	Ig kappa chain V-III region GOL (Rheumatoid faktor)
51	Ig kappa chain V-III region Ti
52	Alpha-2 antiplasmin
53	serum amyloid A protein
54	PRAME family member 2
55	angiotensinogen
56	Stathmin 2
57	Ig kappa chain V-II region TEW
58	hemoglobin subunit Beta
59	Ig kappa chain V-II region GM607

Table: Proteins identified by LC/MS^E in the WGA-binding fraction of normal human plasma

(sorted according to significance scores)

1	Human Serum Albumin*
2	Serotransferrin
3	Fibronectin
4	Kininogen-1 (α_2 -thiol Proteinase Inhibitor)
5	IgG-C _H 1 Region
6	Plasma Protease C1 Inhibitor
7	Haptoglobin
8	Apolipoprotein A-I
9	Hemopexin (Beta-1B-glycoprotein)
10	Ceruloplasmin (Ferroxidase)
11	Complement Factor H
12	Coagulation Factor XII

- 13 Attractin (Mahogany Homolog)
- 14 Pregnancy-zone Protein
- 15 Ig Kappa Chain C Region
- 16 Histidin-rich Glycoprotein
- 17 α_1 -acid Glycoprotein 1 (Orosomucoid-1)
- 18 Carboxypeptidase N Subunit 2
- 19 Inter- α -Trypsin Inhibitor Heavy Chain H4
- 20 IgG-C_H3 Region
- 21 Inter- α -Trypsin Inhibitor Heavy Chain H2
- 22 C4b-binding Protein α Chain (Prolin-rich Protein)
- 23 Haptoglobin-related Protein
- 24 IgA-C_H1 Region
- 25 Keratin, Type II Cytoskeletal 1 (Cytokeratin-1)*
- 26 Ig Lambda Chain C Region
- 27 N-Acetylmuramoyl-L-Alanine Amidase
- 28 α_1 -Antichymotrypsin
- 29 Inter- α -Trypsin Inhibitor Heavy Chain H1
- 30 Complement C1r Subcomponent
- 31 Keratin, Typ I Cytoskeletal 10 (Cytokeratin-10)*
- 32 Hämoglobin Subunit Alpha
- 33 IgA C_H2 Chain C Region
- 34 Clusterin (Apolipoprotein J)
- 35 α_1 -Microglobulin (AMBP)
- 36 Keratin, Typ I Cytoskeletal 9 (Cytokeratin-9)*
- 37 α_2 -HS-Glycoprotein (Fetuin-A)
- 38 IgM Heavy Chain
- 39 Vitronectin
- 40 Complement Factor I
- 41 α_2 -Macroglobulin (Alpha-2-M)
- 42 α_1 -acid Glycoprotein 2 (Orosomucoid-2)
- 43 IgM-C_H1 Region
- 44 Apolipoprotein E (Apo-E)
- 45 Plasma Kallikrein (Kininogenin)
- 46 Lumican (Keratansulfat Proteoglycan Lumican)
- 47 Fibulin-1
- 48 Serum Paraoxonase/Arylesterase 1
- 49 Keratin, Typ II Cytoskeletal 2 Epidermal (Cytokeratin-2e)*
- 50 Phosphatidylcholinsterol Acyltransferase
- 51 Apolipoprotein F (Lipid Transfer Inhibitor Protein)
- 52 Carboxypeptidase B2
- 53 Sulfhydryloxidase 1

54	Keratin, Typ I Cytoskeletal 23 (Cytokeratin-23)*
55	Hämoglobin Subunit Beta*
56	Hämoglobin Subunit Delta*
57	Apolipoprotein-L1
58	Insulin-like Growth Factor (IGF)-binding Protein
59	Hämoglobin Subunit Gamma-1*
60	Hämoglobin Subunit Gamma-2*
61	Apolipoprotein D
62	Chromobox Protein Homolog 5
63	Hämoglobin Subunit Zeta*

. * Non- glycosylated Protein