

Supporting Information

Gold Nanoparticle Probes for Colorimetric Detection of Plasma Galectin-3: A simple and rapid approach

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S1. Characterization of modified Au NPs

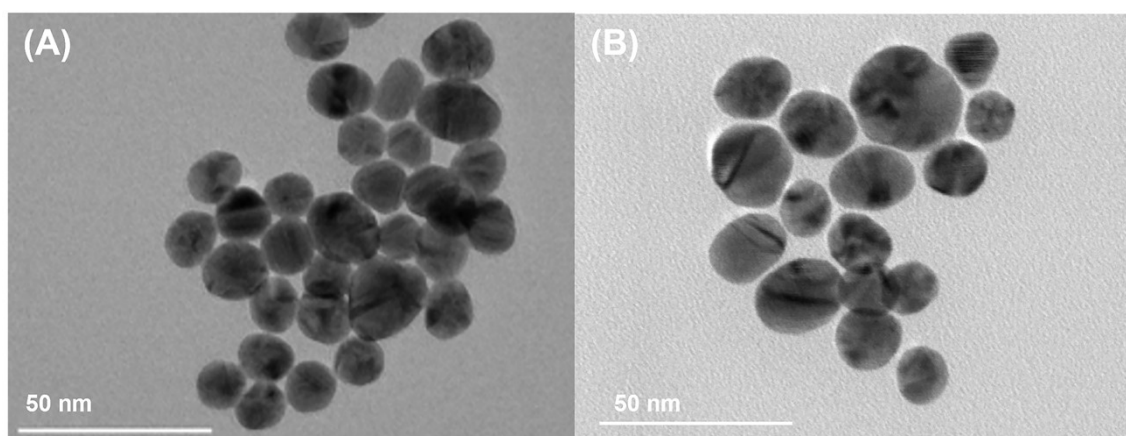


Fig. S1: TEM image of (A) Au NPs; (B) AuNPs@MUDA

Table S1 Values of pH, Zeta potential (ZP), hydrodynamic diameter (HD) and polydispersity index (PDI) of bare and modified Au NPs

Sample	pH	ZP (mV)	HD (nm)	PDI
Au NPs	5.8	-59.3 ± 1.7	24.7 ± 0.5	0.356
AuNPs@MUDA	7.2	-73.1 ± 2.6	65.5 ± 0.4	0.454
AuNPs@MUDA@Ab	7.0	-29.7 ± 0.4	111.3 ± 0.6	0.301
AuNPs@MUDA@Ab@BSA	7.0	-30.5 ± 1.2	103.6 ± 0.1	0.290

The ATR-FTIR spectrum of Ab was acquired to investigate the binding of Ab to AuNPs@MUDA. The ATR-FTIR spectrum of Ab is complex due to the presence of PBS, gelatin, and sodium azide. According to the supplier the Ab was in a PBS solution with 0.1%.

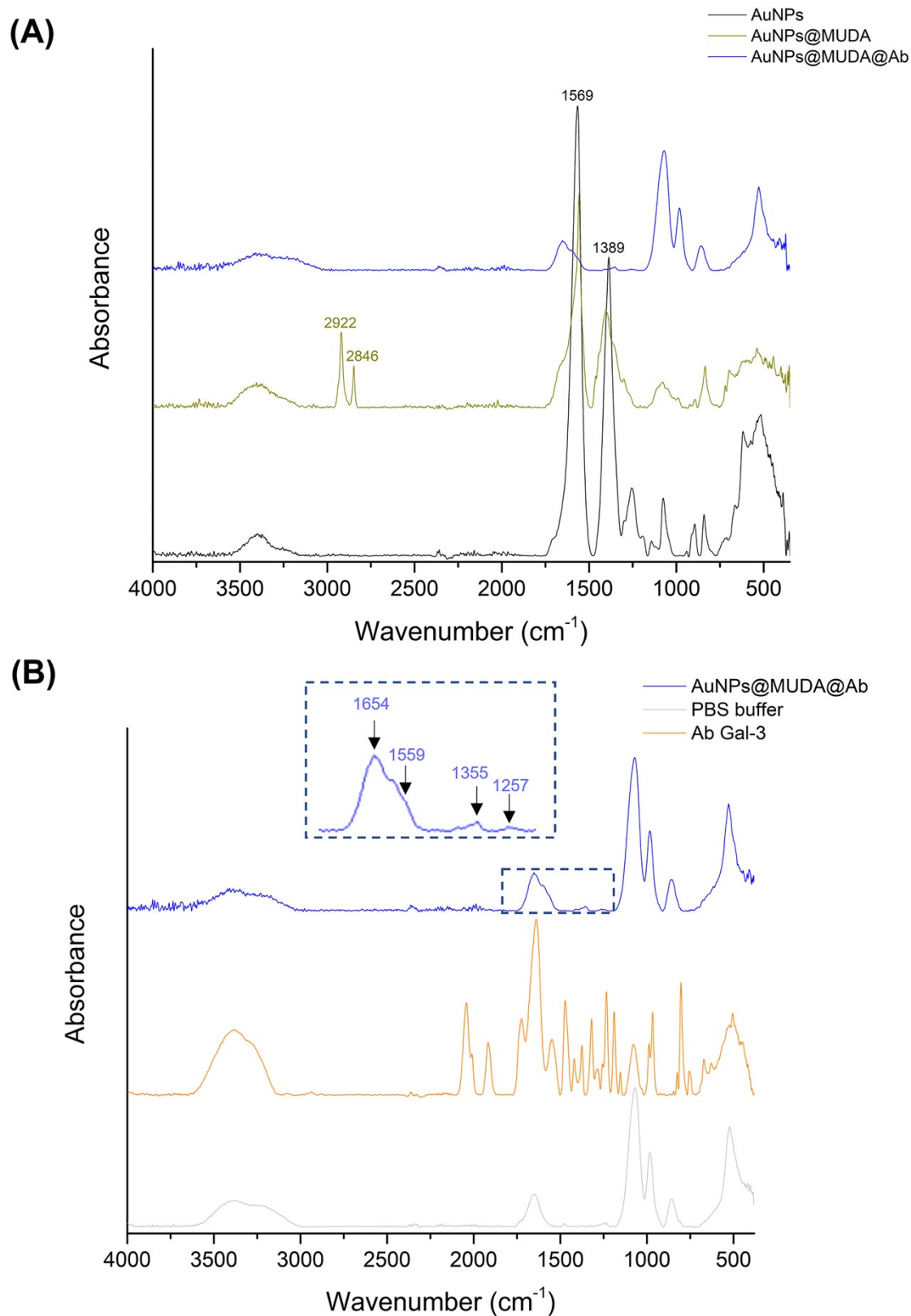


Fig. S2: (A) ATR-FTIR spectra of **(A)** Au NPs, AuNPs@MUDA and Au NPs@MUDA@Ab, and **(B)** PBS buffer, Ab Gal-3 and Au NPs@MUDA@Ab.

S2. Detection of galectin-3 using Au NPs@MUDA@Ab@BSA

Table S2: Hydrodynamic diameter (HD) and polydispersity index (PDI) values of modified AuNPs after incubation with Gal-3 at the concentration of 0, 40 and 160 $\mu\text{g}\cdot\text{L}^{-1}$ in PBS buffer

Galectin-3 ($\mu\text{g}\cdot\text{L}^{-1}$)	HD (nm)	PDI
0	121.9 ± 1.4	0.310
40	332.9 ± 4.6	0.518
160	422.6 ± 3.8	0.708

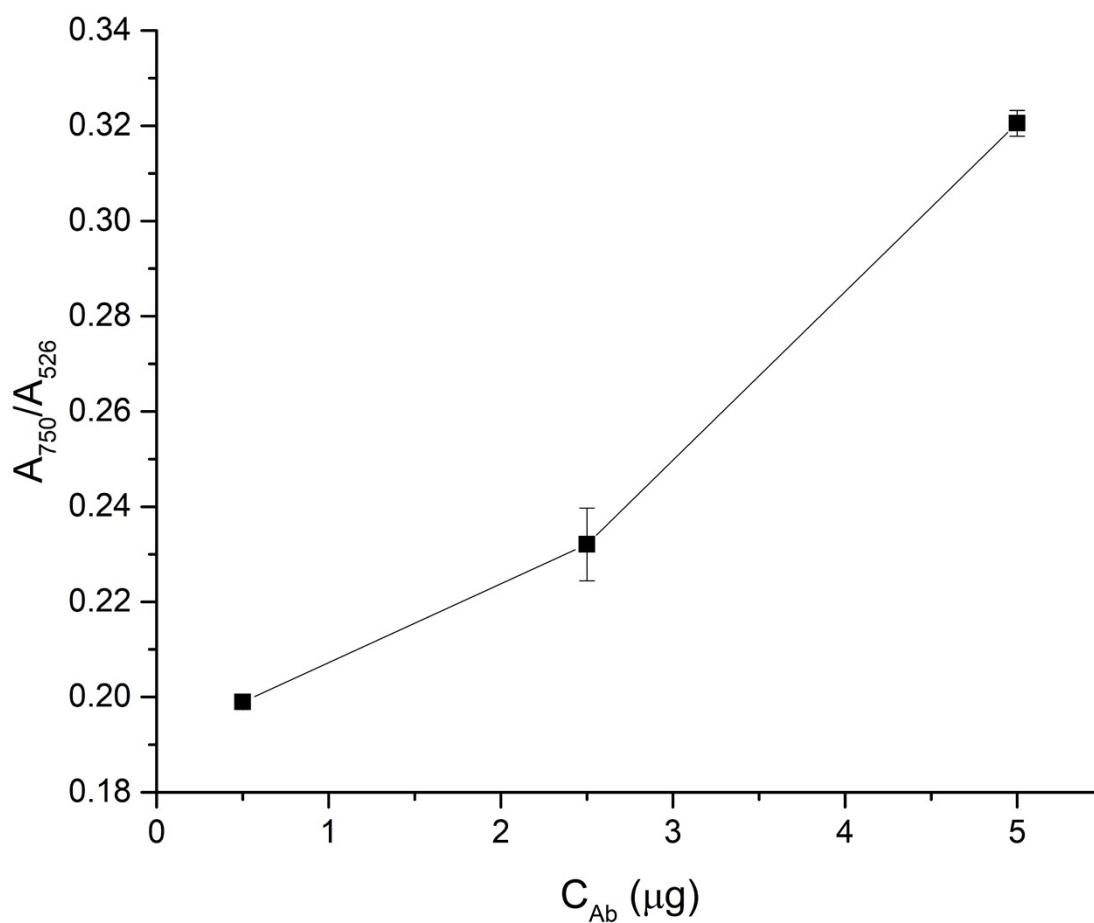


Fig. S3: AR of Au NPs@MUDA@Ab@BSA prepared with 0.5, 2.5 and 5 μg of galectin-3 antibody (Ab) after incubation with Gal-3 ($80 \mu\text{g}\cdot\text{L}^{-1}$ concentration and incubation time of 60 minutes).

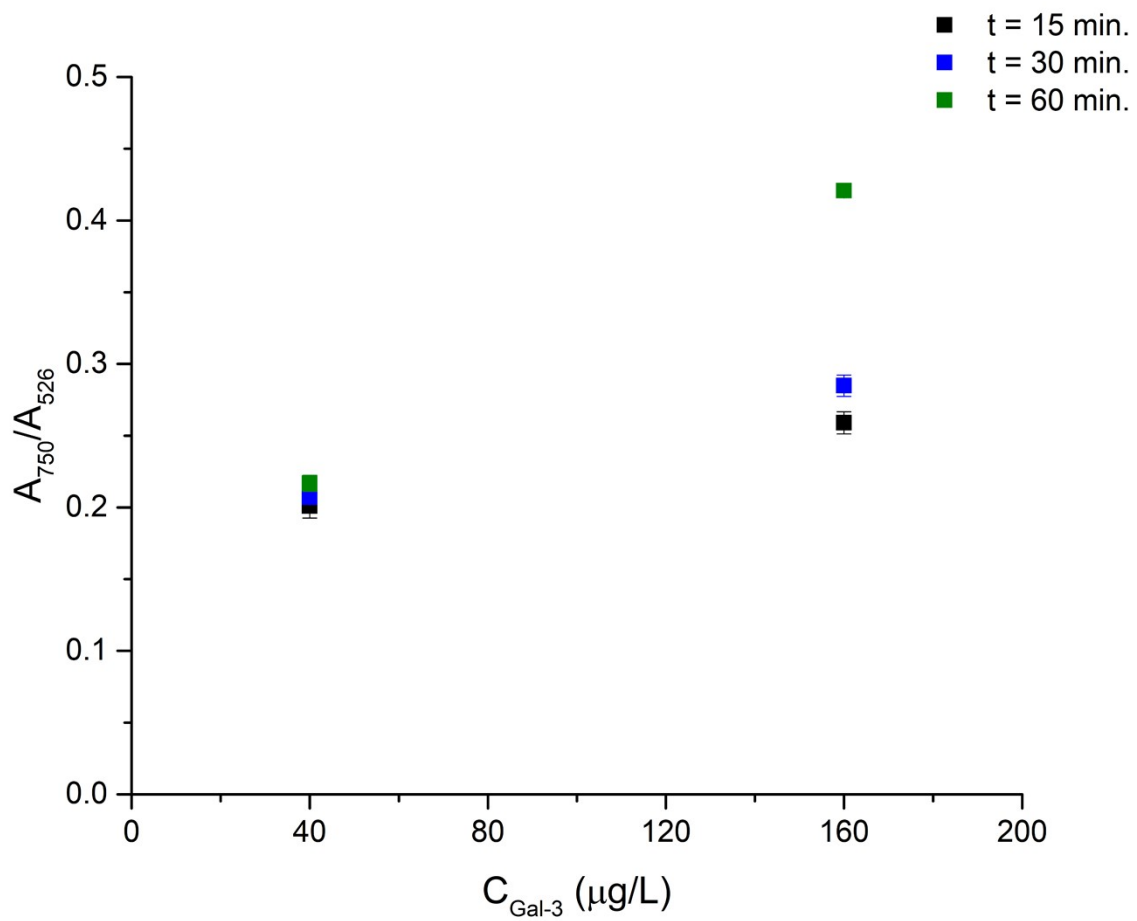


Fig. S4: AR of Au NPs@MUDA@Ab@BSA for 40 and 160 $\mu\text{g}\cdot\text{L}^{-1}$ of galectin-3 after incubation for 15, 30 and 60 minutes.

S3. Galectin-3-antibody interaction

The dissociation constant (k_d) for the AuNPs@MUDA@Ab-Gal-3 was calculated from the variation of A_{750}/A_{526} ratio with over the final concentration of Gal-3 in different solutions, the saturation curves ¹. The k_D value was obtained using the GraphPad Prism software version 8.4.2 and intending that the Gal-3 was able to bind at one specific site, to the antibody. The one site-specific binding equation (**equation S1**) from the software was used. The Y corresponds to the A_{750}/A_{526} ratio, x to the Gal-3 concentrations ($\mu\text{g.L}^{-1}$) and B_{max} is the maximum specific binding.

$$Y = (B_{\text{max}} \cdot x) / (k_d + x) \quad (\text{S1})$$

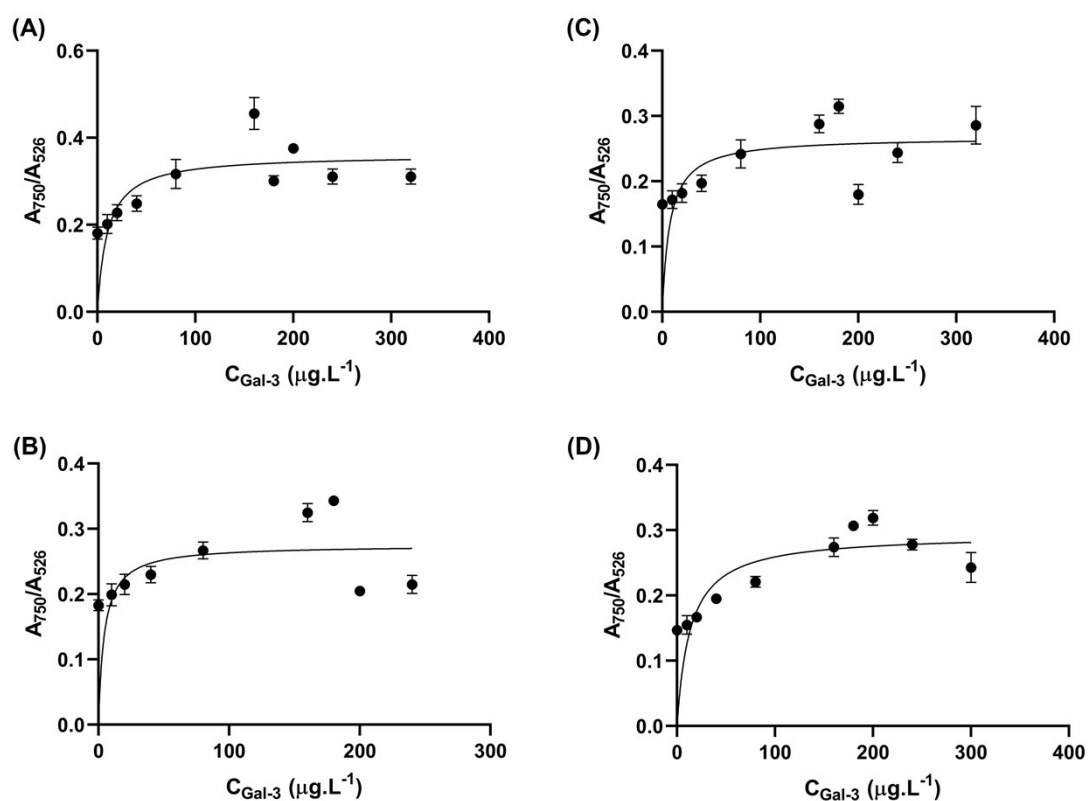


Fig. S5: The fitting curve (solid line) of equation (S1) to determine the dissociation constant k_d of the galectin-3-antibody: **(A)** in buffer, **(B)** diluted saliva, **(C)** 6 protein mixture and **(D)** diluted fetal bovine serum.

S4. Time stability studies

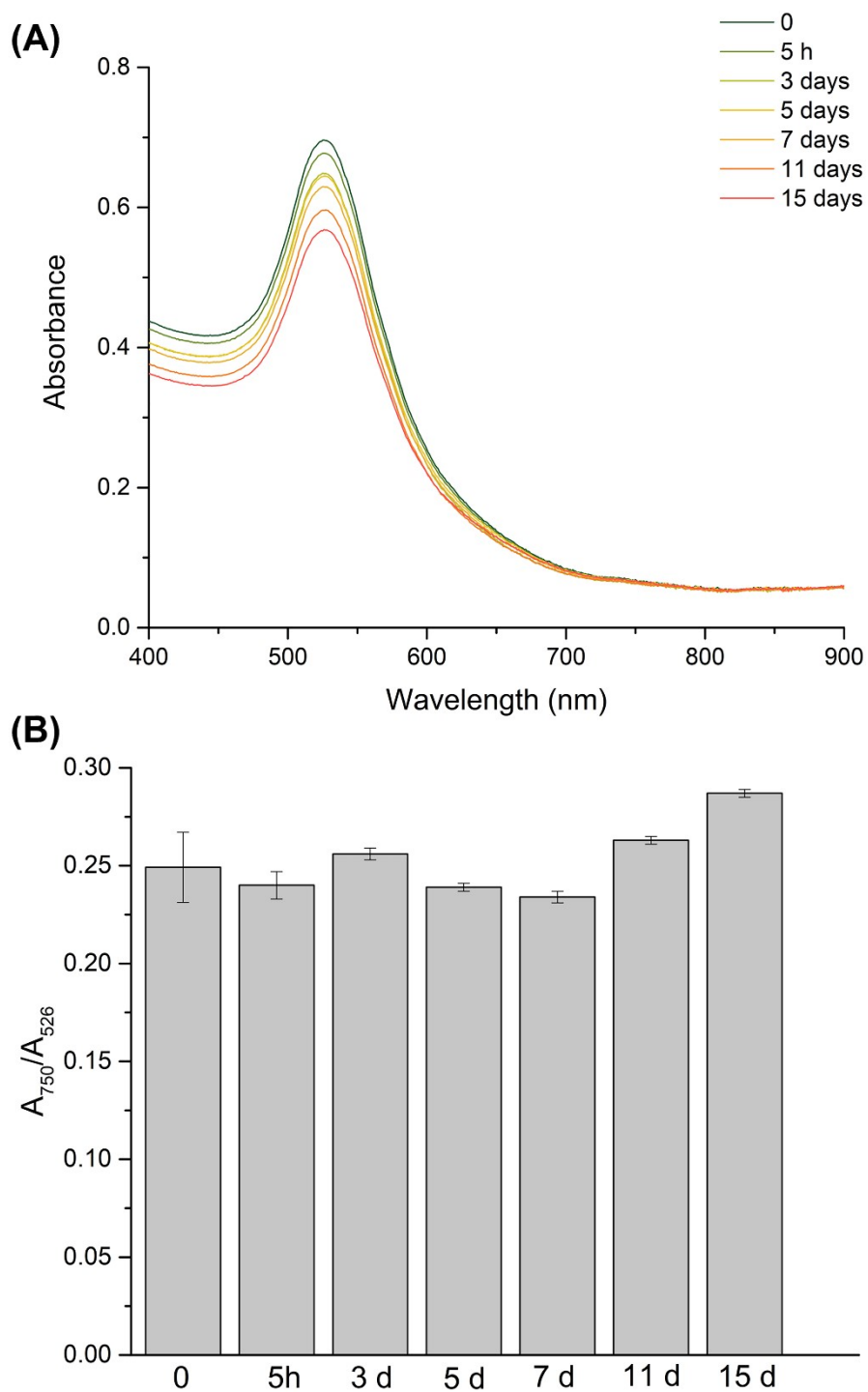


Fig. S6: (A) UV-VIS spectra of AuNPs@MUDA@Ab@BSA colloid, after storage at 4°C for different periods after synthesis (in the absence of galectin-3); (B) AR upon detection of 40 $\mu\text{g}\cdot\text{L}^{-1}$ galectin-3, using AuNPs@MUDA@Ab@BSA probes stored at 4°C after synthesis for 0 hours, 5 hours, 3 days, 5 days, 7 days, 11 days and 15 days.

S5. Plasma samples analysis

Table S3: Concentration of galectin-3 by the developed method, adj. vol. from western and their respective ratio for each analyzed plasma sample

Sample	Proposed method		Western blot	
	C _{Gal-3} (µg.L ⁻¹)	Ratio	Adj.Vol.	Ratio
P0	280.5	1	32.5	1
P1	260.3	1.08	27.7	1.17
C2	143.9	1.95	16.3	1.99
C3	233.6	1.20	24.6	1.32

References

- 1 M. António, R. Ferreira, R. Vitorino and A. L. Daniel-da-Silva, *Talanta*, 2020, **214**, 120868.