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Supporting Information

Electroanalytical Immunotool to Determine Extracellular Matrix Protein Periostin, a Stromal Biomarker of Prognosis in Colorectal Cancer

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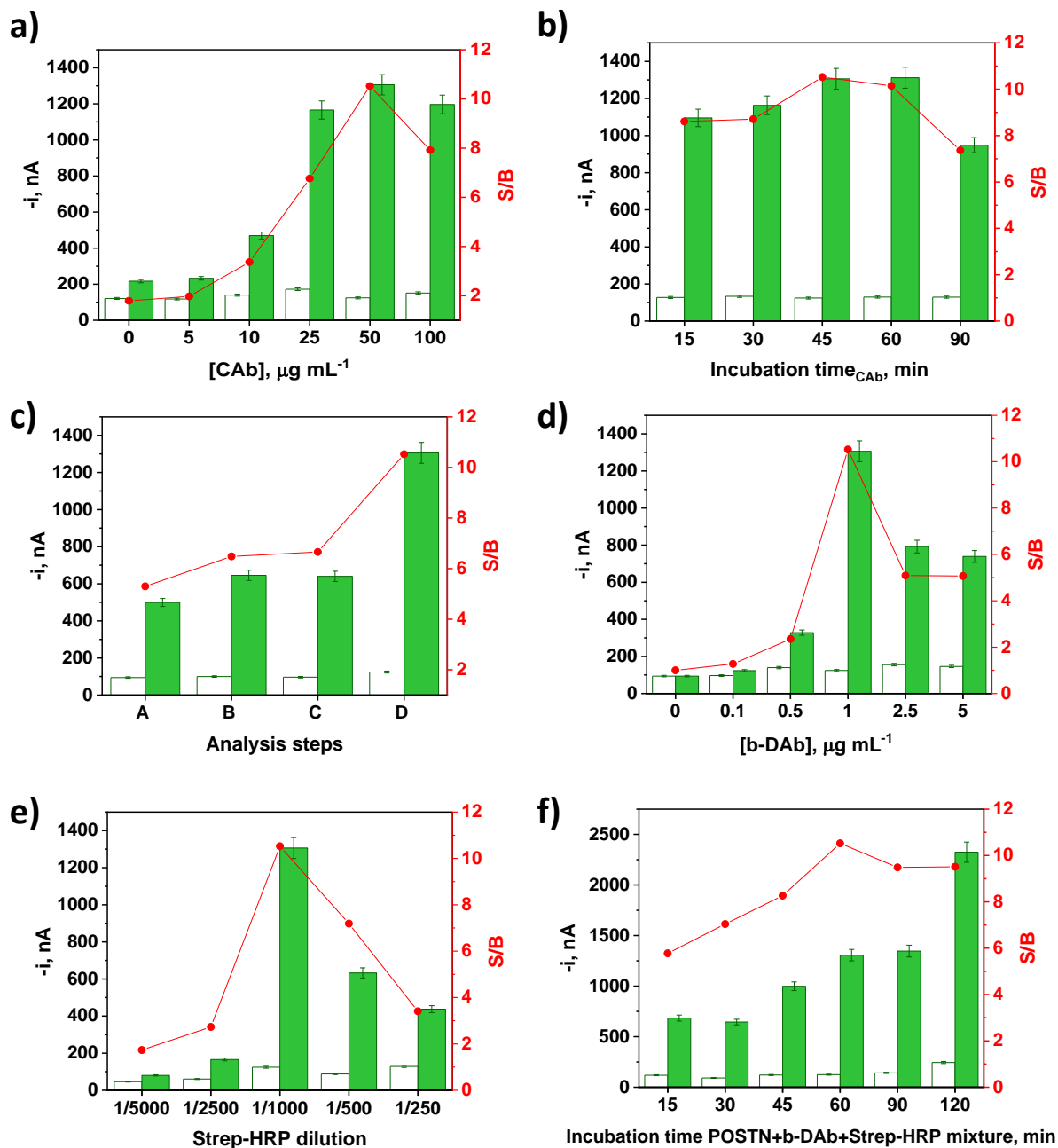


Figure S1. Dependence of the amperometric responses provided by the developed immunoplatfrom for 0.0 (white bars, B) and 10 ng mL^{-1} (green bars, S) of POSTN standards and the resulting S/B, with the CAB concentration (a) and incubation time (b), number of steps to perform the bioassay (c), b-DAB concentration (d), Strep-HRP dilution (e), and incubation time of the CAB-MBs with the POSTN, b-DAB and Strep-HRP mixture solution (f). Error bars were estimated as the SD of three replicates.

Table S1. Protocols tested for the assembly of HRP-labeled sandwich immunocomplexes on CAb-MBs.

Protocol	Successive incubation steps	POSTN	b-DAb	Strep-HRP	Assay time
		(min)			
A	3	60	60	30	150
B	2	60		30	90
C	2	60	60		120
D	1	60			60

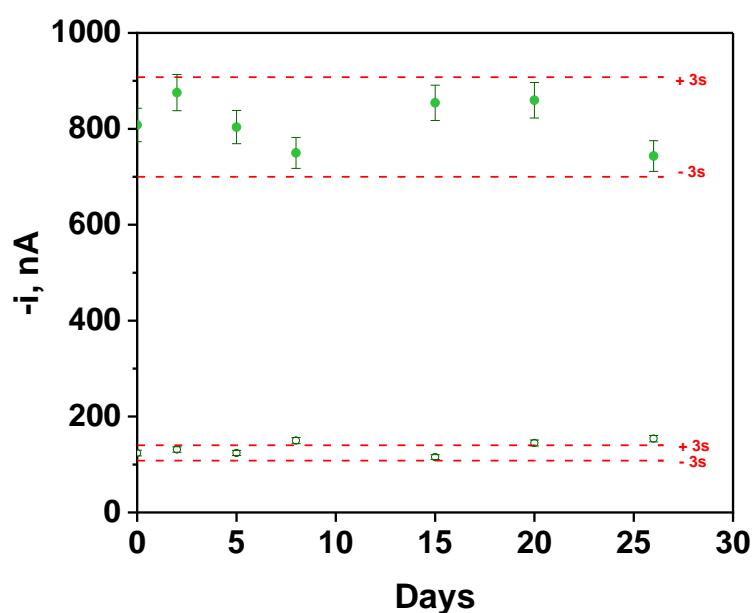


Figure S2. Storage stability of the CAb-MBs immunoconjugates stored at 4 °C in filtered PBS. Amperometric responses provided by the developed immunoplatfrom for 0.0 (empty circles) and 5 ng mL⁻¹ of POSTN standards (filled green circles) using the CAb-MBs conjugates prepared on day 0 and stored as indicated until use. Dashed red lines represent the control limits estimated as $\pm 3 \times \text{SD}$ ($n = 3$) of the measurements recorded on day 0. Error bars were estimated as the SD of three replicates.

Table S2. Comparison of the analytical performance of reported (bio)assays for the determination of POSTN.

Detection Strategy	Technique	L.R.	LOD	Total assay time	Application	Ref.
Optical Sensor	ELISA	3.9–50000 ng mL ⁻¹	--	O/N(CAb) + 240 min	Human serum CRC patients	[1]
Optical Sensor	Photonic Biosensor	100–2500 ng mL ⁻¹	10 ng mL ⁻¹	--	Human serum breast cancer patients	[2]
Optical Sensor	Photonic Biosensor	100–2500 ng mL ⁻¹	10 ng mL ⁻¹	--	Human serum breast cancer patients	[3]
Optical Sensor	Aptamer sensor	0.78–6.25 nM	106.68 pM (Buffer) 463.3 pM (Spiked serum samples)	30 min	Human serum	[4]
ARCHITECT Periostin Immunoassay	CL magnetic immunoassay	5.2–73.3 ng mL ⁻¹	--	18 min	Serum samples from healthy individuals and patients with asthma	[5]
Elecsys® Periostin Immunoassay	ECL immunoassay	10–160 ng mL ⁻¹	4 ng mL ⁻¹	18 min	Human serum	[6]
Label-Free electrochemical immunosensor (CV)	AuNPs/PDA/Sili ca/GCE	0.1–150 ng mL ⁻¹	0.06 ng mL ⁻¹	O/N (AuNPs/PDA/Si lica) + 13 h 20 min	Human serum	[7]

Electrochemical immunoassay (Amperometry)	Sandwich immunoassay implemented on the surface of MBs	0.47–25 ng mL ⁻¹	0.14 ng mL ⁻¹	60 min starting from the blocked CAb-MBs (≈3 h)	Extracts and secretomes of cultured CRC cells and plasma from CRC patients	This work
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AuNPs: gold nanoparticles; CAb: capture antibody; CL: chemiluminescence; CRC: colorectal cancer; CV: cyclic voltammetry; ECL: electrochemiluminescence; GCE: glassy carbon electrode; MBs: magnetic microparticles; O/N: overnight; PDA: polydopamine.

Table S3. Comparison of the analytical performance of sandwich ELISA commercial kits available for the determination of human POSTN.

L.R., ng mL⁻¹	LOD, ng mL⁻¹	Assay time, min*	Application	Ref./web page (accessed nov 24th, 2023)
0.078–5	0.021	90	Cell culture media, plasma, serum	Abcam (ab309314)/ https://www.abcam.com/en-sk/products/elisa-kits/human-periostin-elisa-kit-ab309314
--	0.08	--	Cell culture supernatants, plasma, serum	Sigma – Aldrich (RAB1075)/ https://www.sigmaaldrich.com/ES/es/product/sigma/rab1075
0.078–5	0.031	210	Serum, plasma and other biological fluids	CymitQuimica (EK-ELK3069)/ https://cymitquimica.com/es/productos/EK-ELK3069/human-postnperiostin-elisa-kit/
15.6–1000	0.098	--	Serum, plasma, tissue homogenates, cell culture supernatants and other biological fluids	Gentaur (812-E1993Hu)/ https://gentaur.es/sunlong/producto-human-postn-periostin-elisa-kit-id-5886926
0.062–4	0.03	215	Cell culture supernatants, serum, plasma	MyBioSource (MBS824679)/ https://www.mybiosource.com/postn-human-elisa-kits/periostin/824679
0.5–150	0.25	90	Serum, plasma, cell culture supernatants	BT LAB

				(E3226Hu)/ https://www.bt-laboratory.com/index.php/Shop/Index/productShijiheDetail/p_id/981/cate/kit.html
				ElabScience
0.16–10	0.09	210	Serum, plasma and other biological fluids	(E-EL-H6160)/ https://www.elabscience.com/p-human_postn_osf_2_periostin_elisa_kit-552576.html
				ThermoFisher (EHPOSTN)/
0.08–60	0.08	285	Plasma, serum, supernatant	https://www.thermofisher.com/elisa/product/Periostin-Human-ELISA-Kit/EHPOSTN
				Abbexa
0.078–5	0.031	180	Serum, plasma and other biological fluids	(abx152708)/ https://www.abbexa.com/human-periostin-elisa-kit-p-52595
				Cusabio
6.25–400	1.56	300	Serum, plasma, tissue homogenates	(CSB-E16444h)/ https://www.cusabio.com/ELISA-Kit/Human-periostinosteoblast-specific-factor-2-POSTN-ELISA-kit-97429.html
				R&D Systems (DY3548B)/
0.0625–4	--	280 min	Cell culture supernatants, serum, and plasma	https://www.rndsystems.com/products/human-periostin-osf-2-duoset-elisa_dy3548b

**Starting from the CAb-precoated ELISA plate.*

Table S4. Slope values (absolute values in nA ng⁻¹ mL) calculated from the calibration plots constructed with the developed bioplatfrom for the amperometric determination of POSTN standards prepared in buffered solutions and in representative biological samples.

POSTN standards prepared in	Slope	t _{exp} **	t _{tab} (95 %, 2, 2 tails)**
Buffered solutions	118 ± 10	--	
1.0 µg SW480 extract	404 ± 61	4.632	
5-times diluted SW480 secretome	92 ± 5	2.301	4.303
3-times diluted KM12C secretome	46 ± 8	5.638	
25-times diluted plasma from healthy subject	307 ± 35	5.210	

**Estimated as described in^[8].

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