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Supporting information

Ultrasensitive detection of seventeen chemicals simultaneously using paper-based sensors

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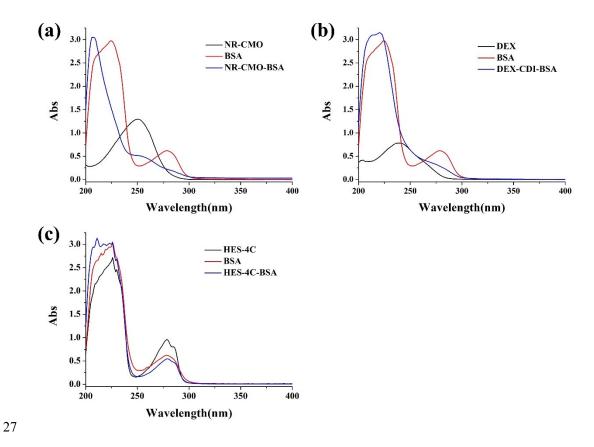
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7 Monoclonal antibody preparation

For preparing the anti-NR mAb, eight female BALB/c mice (6-8 weeks old) were 8 prepared, and then immunized subcutaneously with NR-CMO-BSA. The process are 9 as follows. The first immunizing does consisted of 100µg of antigen as an emulsion of 10 PBS and FCA. Four sequential boosters were administered at 3-week intervals with 11 50µg of immunogen emulsified in FIA. After each booster, the serum collected form 12 the tail vessel of each mouse were detected for the antibody specificity by ic-ELISA. 13 The mouse with the highest title and the best specificity to DEX was chosen to inject 14 intraperitoneally with 25µg of immunogen dissolved in 100µL normal saline. 15

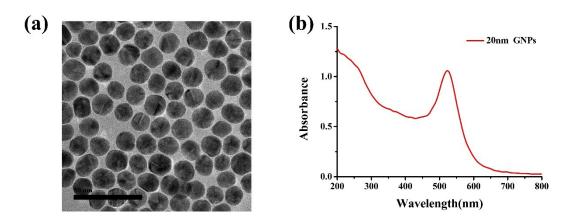
Myeloma cells were fused with the spleen cells collected from the BALB/c mouse 16 whose antiserum had the lowest 50% inhibitory concentration (IC_{50}) and the highest 17 titer, using the standard procedure. The supernatant from the hybridoma was detected 18 with an ic-ELISA. We subcloned the positive hybridoma showing the expected 19 inhibition. After three rounds of subcloning with the limiting dilution method, we 20 isolated the target cell strain producing the antibody that best inhibited NR. The strain 21 was expanded, cultured, and injected intraperitoneally into 8-10-week-old BALB/c 22 mice. The antibody was purified from the mouse ascites with the caprylic acid-23 ammonium sulfate precipitation method and dialyzed against PBS at 4 °C for 3 days. 24

25 The anti-DEX mAb and the anti-HES mAb were produced similarly under their26 individually optimized conditions.



28 Fig. S1 The UV spectra of coating antigens. (a) Confirmation of NR-CMO-BSA; (b)

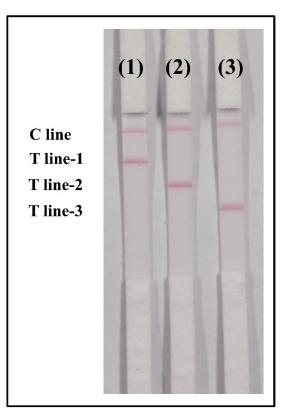
29 Confirmation of DEX-CDI-BSA; (c) Confirmation of HES-4C-BSA.



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- 32 Fig. S2 Characterization of GNPs solution: (a) TEM images; (b) UV-visible spectra,
- 33 and the maximum absorbance of GNPs solution is 523nm.

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- **Fig. S3** Specificity of each test line. (1) Only anti-NR mAb-GNP conjugates; (2) Only
- 37 anti-DEX mAb-GNP conjugates; (3) Only anti-HES mAb-GNP conjugates.

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Abbreviation	Full name
NR-CMO-BSA	Nandrolone- Carboxymethoxylamine hemihydrochloride-
	Bovine serum albumin conjugate
DEX-CDI-BSA	Dexamethasone- N,N'-carbonyldiimidazole- Bovine serum
	albumin conjugate
HES-4C-BSA	Hexestrol-4-bromobutyrate- Bovine serum albumin
	conjugate
anti-NR-mAb	The monoclonal antibody against Nandrolone and its
	analogues
anti-DEX-mAb	The monoclonal antibody against Dexamethasone and its
	analogues
anti-HES-mAb	The monoclonal antibody against Hexestrol and its
	analogues
GNPs	Colloidal gold nanoparticles
LC-MS	Liquid chromatography-mass spectrometry

39 Table S1: The abbreviation and its full name.