

Electronic Supplementary Information (ESI)

**A novel signal amplification strategy for high specific and nonenzymatic
isothermal electrochemiluminescence detection of tumour markers**

Yan Liu^a, Xin Guo^b, Zhijin Fan^c, Yuhui Liao^c, Ying Yu^a, Manli Guo^a, Yujuan Cao^a

^{*}, Debin Zhu^{a*}

^a Guangzhou Key Laboratory of Analytical Chemistry for Biomedicine, School of Chemistry, South China Normal University, Guangzhou 510006, China

^b Centre for Optical and Electromagnetic Research, South China Academy of Advanced Optoelectronics, South China Normal University, Guangzhou 510006, China

^c Program of Infection and Immunity, the Fifth Affiliated Hospital of Sun Yat-sen University, Zhongshan School of Medicine, Sun Yat-sen University, Guangdong 510080, China

* Corresponding author.

E-mail: caoyj@scnu.edu.cn, zhudb@scnu.edu.cn

Tel: +86-20-39310257

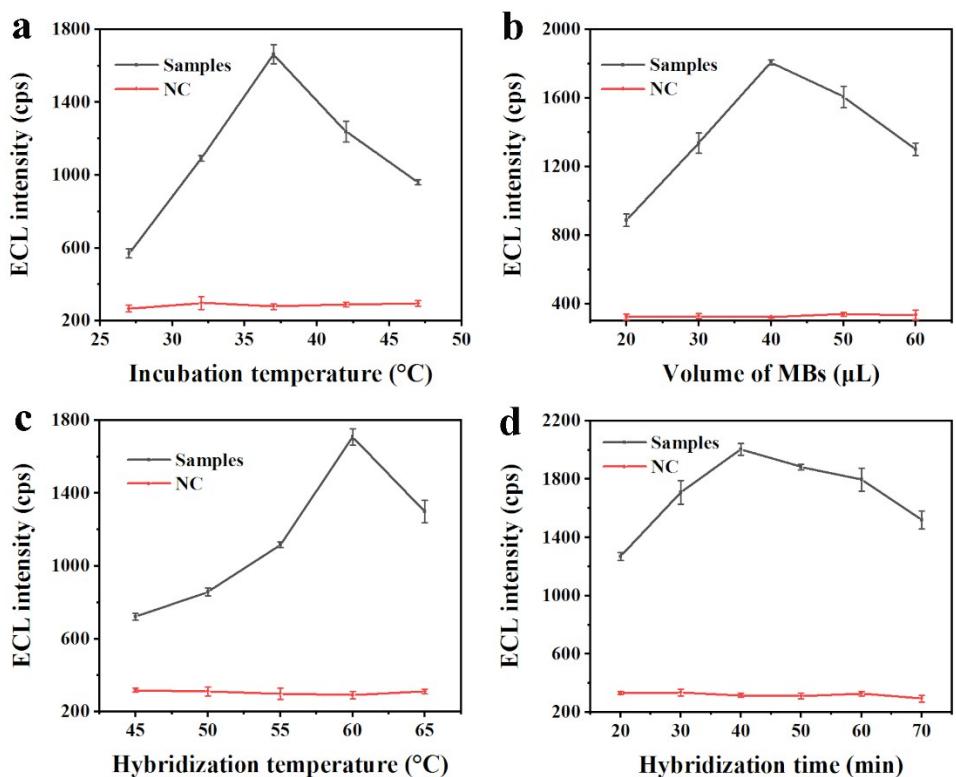


Figure S1 Experimental parameters optimization of incubation temperature (a), volume of MBs (b), hybridization temperature (c), and hybridization time (d).

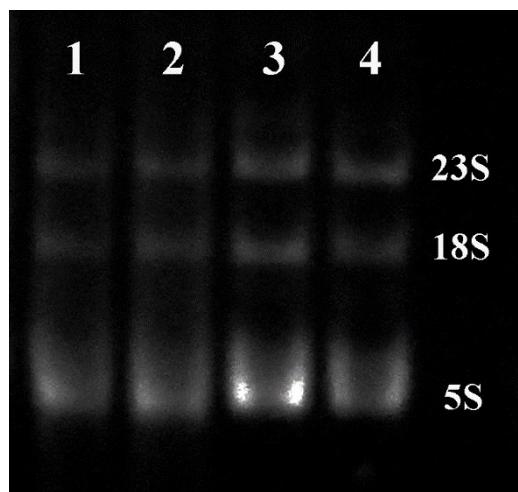


Figure S2 Total RNA isolation

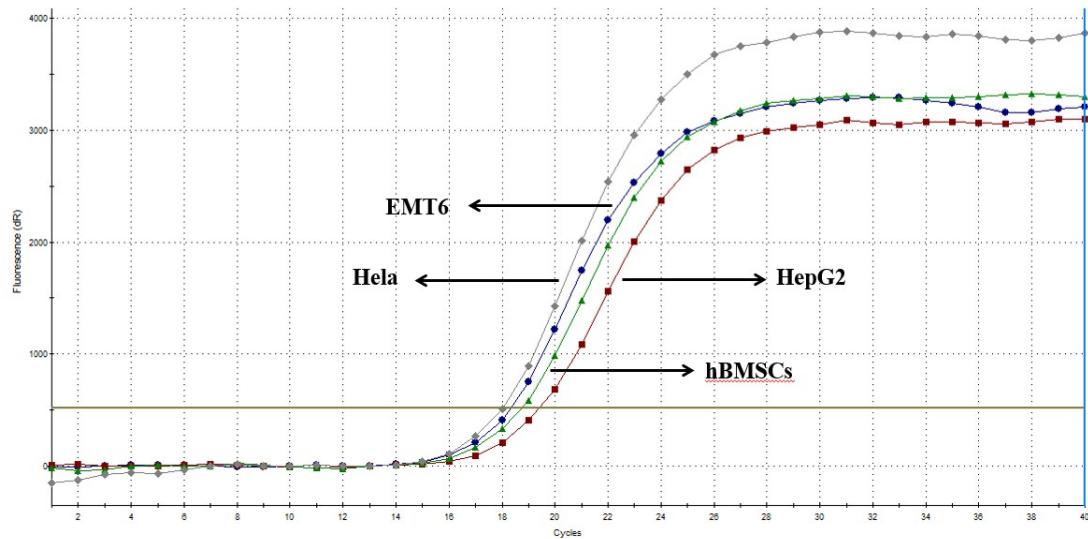


Figure S3 Quantitative real-time PCR experiment for miRNA21 detection in various cell lines

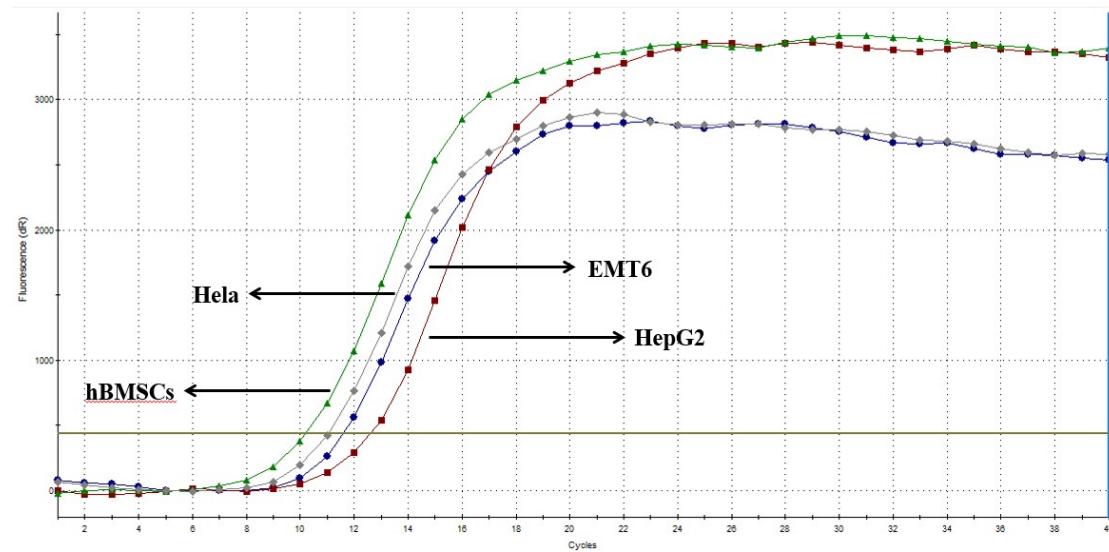


Figure S4 Quantitative real-time PCR experiment for U6 small nuclear RNA (snRNA) detection in various cell lines. U6 small nuclear RNA (snRNA) detection in various cell lines. U6 small nuclear RNA was employed as the universal endogenous control and the relative expression was calculated by the equation: Fold change = $2^{-\Delta\Delta Ct}$

Table S1. Sequences of microRNAs and probes

Name	Sequence (5'-3')
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-20a	UAA AGU GCU UAU AGU GCA GGU AG
miRNA-20b	CAA AGU GCU CAU AGU GCA GGU AG
miRNA-18b	UAA GGU GCA UCU AGU GCA GUU AG
let-7a	UGA GGU AGU AGG UUG UAU AGU U
NC	UUG UAC UAC ACA AAA GUA CUG
Pre-capture probe	Biotin-ACTAGACCGAGTAGGTTTTCTGATAAGCTA
Blocker	CCTACTCGGTCTAGT
Capture probe	TCAACATCAGTTTCATACGCGCGACGATACGGC AGGTGCCGGGATCCCTGTTGCTTAGGTGCCGGGATCCCT
LY-Preamp	GTTGCTTAGGTGCCGGGATCCCTGTTGCTTGCCGTATC GTCGCGCGTATG GCAACAGGGATCCGGCACCTTCGTGAACCATGCCGC
LY-AMP	GACTGATTCTGTGAACCATGCCGCACTGATTCTGTGAAC CATGCCGCAGTGA
TBR-LP for linear DNA	NH ₂ -GCCGTATCGTCGCGCGTATG
TBR-LP	NH ₂ -TCAGTCGCGGCATGGTTCACG

Table S2. The sequences for stem-loop qRT-PCR

Name	Sequence (5'-3')
Reverse transcriptase hairpin	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACTG GATACGACTCAACA
Forward primer	GCCCGCTAGCTTATCAGACTGATG
Reverse primer	GTGCAGGGTCCGAGGT
U6 Forward primer	CTCGCTTCGGCAGCACA
U6 Reverse primer	AACGCTTCACGAATTGCGT

Table S3. The Ct value of stem-loop qRT-PCR for various cell lines

Name	Ct _{miRNA21}	Ct _{U6}	-ΔΔCt	fold change	Relative expression
hBMSCs	18.77	10.80	1.01	0.50	1
EMT6	18.41	11.42	0.017	0.99	1.98
HeLa	17.99	11.11	-0.093	1.06	2.12
HepG2	19.38	12.76	-0.36	1.28	2.56