Supporting Information

Truly Immobilization-Free Diffusivity-Mediated Photoelectrochemical Biosensing Strategy for Facile and Highly Sensitive MicroRNA Assay

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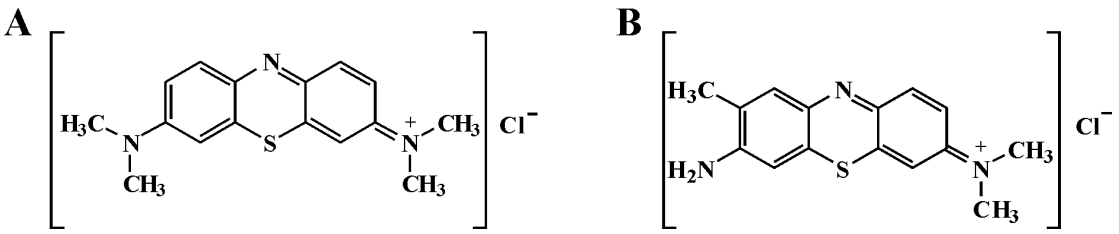

Figure S1. The chemical structures of (A) methylene blue and (B) toluidine blue.

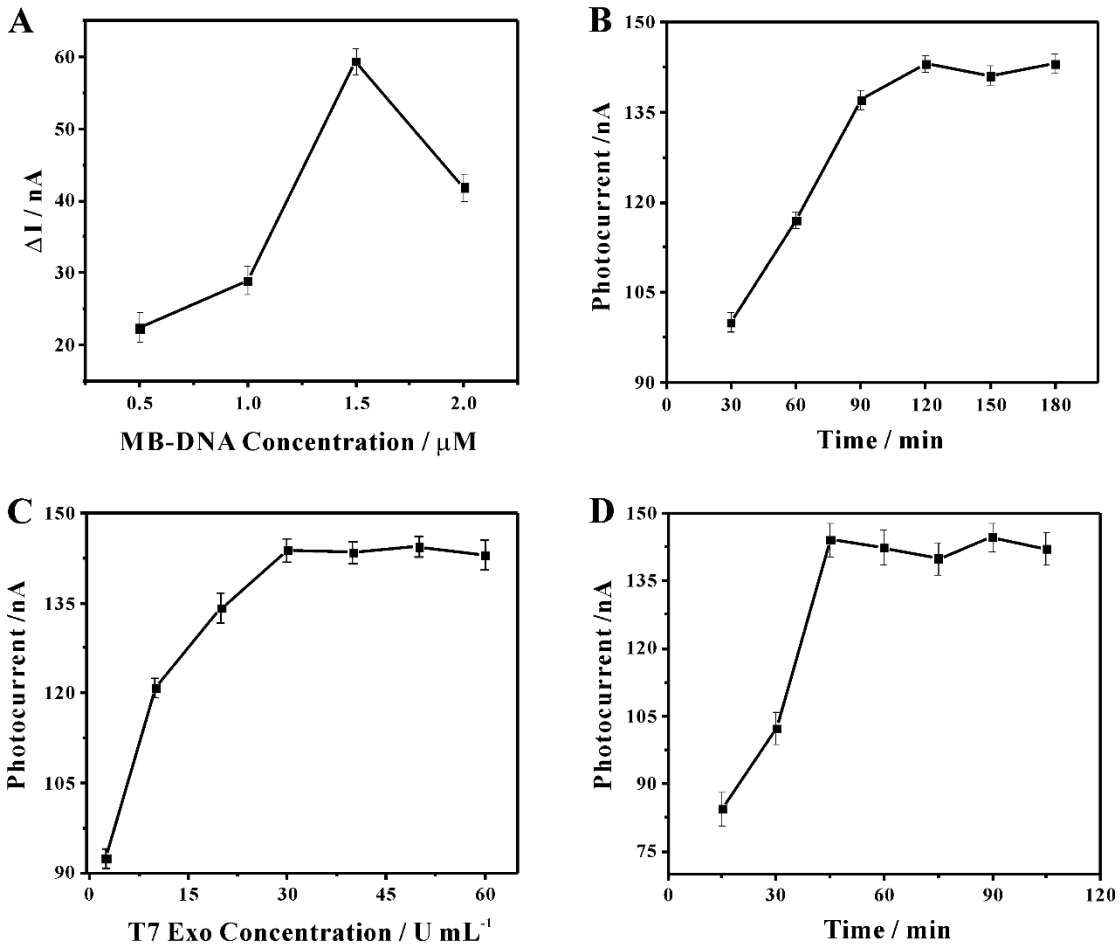

Figure S2. Experimental condition optimization: (A) The photocurrent change ΔI versus the MB-DNA concentration (0.5, 1.0, 1.5 and 2.0 μM), where $\Delta I = I - I_0$, in which I_0 is the photocurrent of the reaction system in the absence of miRNA-155, and I is the photocurrent in the presence of 100 fM miRNA-155. The concentration of T7 exonulcease was 30 U/mL; (B) The photocurrent versus the hybridization time between MB-DNA and miRNA ranging from 30 to 180 min, and the concentrations of MB-DNA, miRNA-155 and T7 Exo were 1.5 μM, 100 fM and 30 U mL^{-1}, respectively. (C) The photocurrent versus T7 Exo concentration (2.5, 10, 20, 30, 40, 50 and 60 U mL^{-1}), in the presence of 1.5 μM MB-DNA, 100 fM miRNA-155 and 0.1 M AA; (D) The photocurrent versus the T7 Exo reaction time (15, 30, 45, 60, 75, 90, and 105 min), and the concentrations of T7 Exo, MB-DNA, miRNA-155 and AA were 30 U mL^{-1}, 1.5 μM, 100 fM and 0.1 M, respectively. The error bars represent the standard deviation of five repetitive measurements.

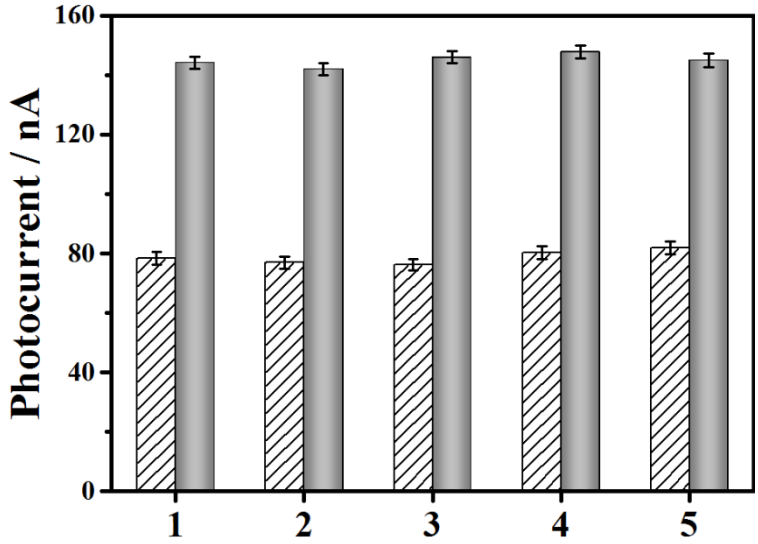

Figure S3. Photocurrents obtained on five different bare ITO electrodes in the absence (dashed columns) and in the presence of 100 fM miRNA-155 (grey columns), where the concentrations of MB-DNA, T7 Exo and AA were 1.5 μM, 30 U mL^{-1} and 0.1 M, respectively. The error bars represent the standard deviation of five repetitive measurements.

Table S1. Sequence information of the oligonucleotides used in this work.

Name	Sequence (from 5´to 3´)
MB-DNA (methylene blue-labeled signal probe)	5′-MB-AC CCC TAT CAC GAT TAG-3′
Non-labeled DNA	5′-AC CCC TAT CAC GAT TAG-3′
miRNA-155 (target) [a]	5′-UUA AUG CUA AUC GUG AUA GGG GU-3′
miRNA-141	5′-UAA CAC UGU CUG GUA AAG AUG G-3′
miRNA-143	5′-UGA GAU GAA GCA CUG UAG CUC A-3′
miRNA-199a	5′-ACA GUA GUC UGC ACA UUG GUU A-3′

[a] For miRNA-155, the underlined letters represent the sequences complementary to either MB-DNA or Non-labeled DNA.

Table S2. Comparison of the analytical performance for miRNA assay by our strategy and those reported in literature.

Detection Method	Linear Detection Range	Limit of Detection	Reference
Immobilization-free photoelectrochemistry	80 aM ~ 10 pM	27 aM	This work
Photoelectrochemistry	0.25 fM ~ 25 pM	83.3 aM	1
Photoelectrochemistry	1 fM ~ 100 pM	0.2 fM	2
Photoelectrochemistry	50 fM ~ 100 pM	34 fM	3
Photoelectrochemistry	350 fM ~ 5 nM	153 fM	4
Electrochemistry	5 fM ~ 50 pM	1.92 fM	5
Electrochemistry	2 fM ~ 1 nM	2 fM	6
Electrochemistry	20 fM ~ 50 pM	5.36 fM	7
Electrochemistry	10 fM ~ 1 nM	10 fM	8
Electrochemistry	1 pM ~ 10 nM	0.26 pM	9
Electrochemiluminescence	100 aM ~ 100 pM	22 aM	10
Electrochemiluminescence	0.5 fM ~ 50 fM	0.24 fM	11
Electrochemiluminescence	1 fM ~ 100 pM	0.3 fM	12

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