Electronic Supplementary Material (ESI) for Analyst.

SUPPLEMENTARY INFORMATION

A SERS Approach for Rapid Detection of microRNA-17 in the Picomolar Range

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Absorption Spectra of Silver Nanoparticle Colloid. The absorption spectra, Figure S1, was used to identify the location of the absorption maximum. The nanoparticle concentration was calculated using Beer– Lambert's Law ($A = c\varepsilon_{\lambda}l$) based on the intensity of the absorption maximum at 408 nm. The absorption spectra were also used to confirm nanoparticle functionalization with ssDNA. The absorption maximum of the functionalized AgNPs occurred at 410 nm, a slight shift from the bare AgNPs at 408 nm.

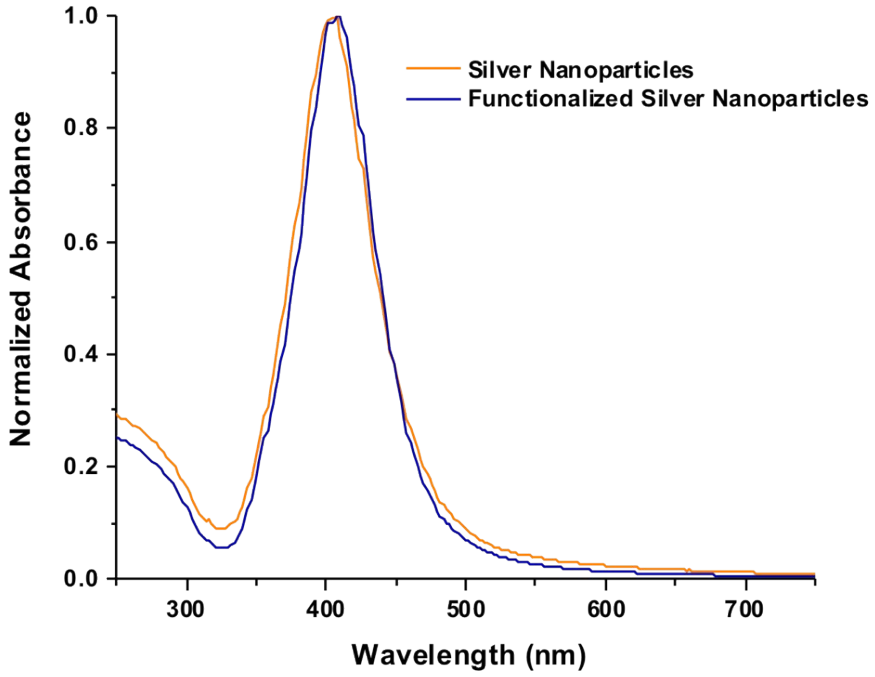

Figure S1. The absorption spectra of bare AgNPs and AgNPs functionalized with ssDNA capture probes.

TEM Images of Synthesized Silver Nanoparticle Colloid. The synthesized AgNPs were further characterized using a Transmission Electron Microscope (TEM). TEM images relay information regarding nanoparticle morphology and were used to confirm the shape and size of the synthesized AgNPs prior to conjugation.

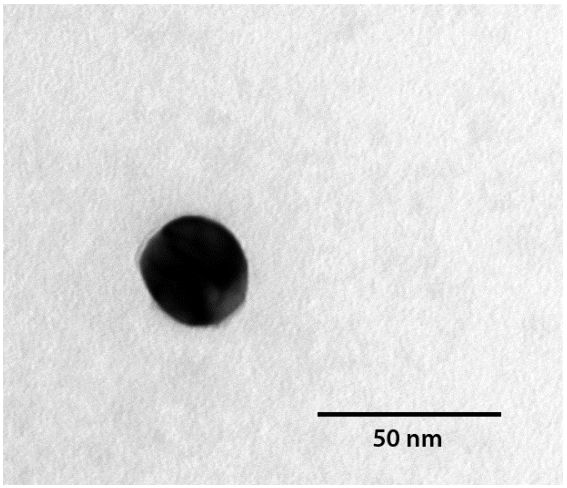

Figure S2. TEM image of synthesized AgNPs

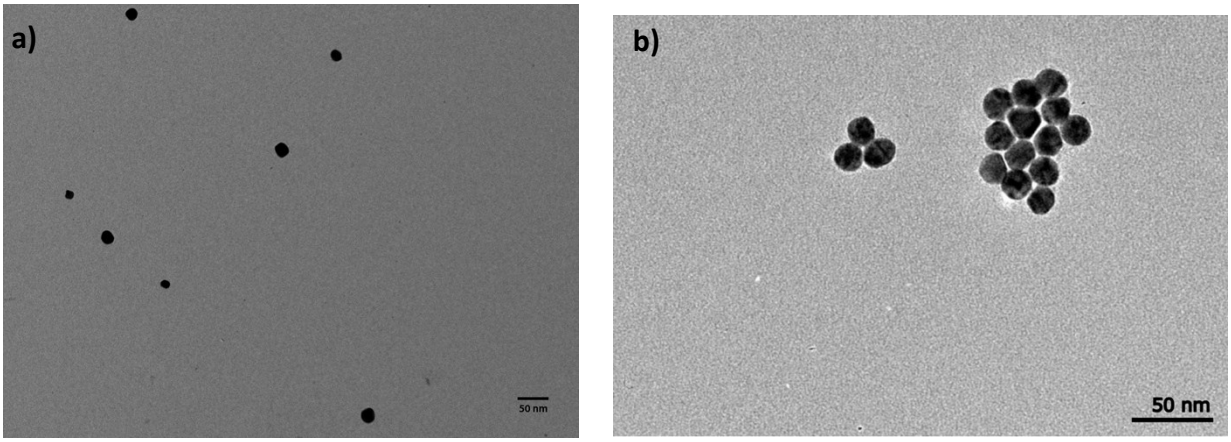

Figure S3. TEM images of (a) functionalized AgNPs without the presence of miRNA-17 and (b) the AgNPs plasmonic aggregates formed in the presence of the target analyte, miRNA-17. The images were obtained by combining the synthesized nanoparticle detection assay (AgNPs conjugated with Probe 1 and labelled with MGITC + AgNPs conjugated with Probe 2) with or without miRNA-17 target and allowing the solution to react at room temperature. The resultant aggregates that formed in response to miRNA-17 varied in the number of nanoparticles per cluster. Aggregates ranges from 2 nanoparticles per cluster to an upward amount of 15 nanoparticles per cluster.

Limit of Detection (LOD) Calculations. The LOD was calculated using the SERS intensity of the blank and its standard deviation. The signal intensity of the LOD (I_{Limit}) was determined using Equation S1. Table S1 provides a summary of intensity values used to calculate the SERS signal intensity of the LOD.

(Equation S1) $$I_{Limit} = I_{Blank} + 3 * SD_{Blank}$$

Table S1. The intensity limit calculations of the blank for the characteristic peaks of MGITC

	LIMIT OF DETECTION (LOD) CALCULATIONS USING LINEAR REGRESSION ANALYSIS					
	DETECTION ASSAY IN PBS			*DETECTION ASSAY IN 20% (v/v) BOVINE SERUM*		
	I_{Blank}	SD_{Blank}	I_{Limit}	I_{Blank}	SD_{Blank}	I_{Limit}
1177 cm^{-1}	3.20E+04	1.50E+03	3.65E+04	6.80E+04	1.10E+04	1.01E+05
1220 cm^{-1}	9.50E+03	4.80E+02	1.09E+04	1.10E+04	3.10E+03	2.03E+04
1290 cm^{-1}	9.20E+03	6.70E+02	1.12E+04	2.40E+04	3.60E+03	3.48E+04
1586 cm^{-1}	1.50E+04	1.80E+02	1.55E+04	3.30E+04	5.30E+03	4.89E+04
1618 cm^{-1}	2.50E+04	1.60E+03	2.98E+04	4.60E+04	7.50E+03	6.85E+04

The I_{Limit} was then used to specify the miRNA-17 concentration associated with the LOD. The representative equations that relate miRNA-17 concentration (x) and SERS signal intensity (y) for each of the characteristic peaks was used to determine the corresponding target concentration for the LOD. Linear regression analysis was used to relate signal intensity to target concentration for the I_{LIMIT} (Equation S2). Table S2 provides the values for the equation constants and the calculated target concentration for and intercept calculated for each peak.

(Equation S2) $$y = m * log(x) + k$$

Table S2. The target concentrations for the LOD calculated for the five characteristic peaks analysed

	LIMIT OF DETECTION (LOD) CALCULATIONS USING LINEAR REGRESSION ANALYSIS							
	DETECTION ASSAY IN PBS				*DETECTION ASSAY IN 20% (v/v) BOVINE SERUM*			
	y	k	m	LOD (M)	y	k	m	LOD (M)
1177 cm^{-1}	3.65E+04	3.60E+05	2.66E+04	6.89E-13	1.01E+05	7.42E+05	5.29E+04	7.63E-13
1220 cm^{-1}	1.09E+04	9.33E+04	6.84E+03	9.10E-13	2.03E+04	1.17E+05	8.38E+03	2.89E-12
1290 cm^{-1}	1.12E+04	9.24E+04	6.70E+03	7.62E-13	3.48E+04	2.22E+05	1.52E+04	4.83E-13
1586 cm^{-1}	1.55E+04	1.60E+05	1.13E+04	1.64E-13	4.89E+04	3.83E+05	2.64E+04	2.21E-13
1618 cm^{-1}	2.98E+04	2.16E+05	1.48E+04	2.62E-13	6.85E+04	5.20E+05	3.69E+04	5.81E-13

Dose-Response Analysis Using Hill Equation. The SERS intensity at various miRNA-17 concentrations was fit to a dose-response curve using the Hill Equation (Equation S3). However, the Hill1 Equation is only valid for intensities within the range defined by the START and END values (Table S3). As a result, the target concentrations calculated based on the Hill1 equation were undefined for the intensity values at the LOD. To determine the representative Hill equation, the miRNA-17 detection assay was tested using a concentration range of 0.01 pM to 10000 pM. The SERS spectra obtained for this entire range is provided in Figure S4.

(Equation S3)

$$y = START + (END - START)\frac{x^n}{k^n + x^n}$$

Table S3. Hill equation coefficients for the characteristic peaks of MGITC

	DOSE-RESPONSE ANALYSIS USING HILL EQUATION											
	DETECTION ASSAY IN PBS						*DETECTION ASSAY IN 20% (v/v) BOVINE SERUM*					
	k (pM)	**n**	**COD**	**R^2**	**START**	**END**	**k (pM)**	**n**	**COD**	**R^2**	**START**	**END**
1177 cm^{-1}	107	0.958	0.995	0.999	4.64E+4	1.34E+5	8.58	1.15	0.997	0.994	8.94E+4	2.34E+5
1220 cm^{-1}	85	0.875	0.996	0.993	1.22E+4	3.50E+4	15.1	0.674	0.999	0.999	1.13E+4	4.19E+4
1290 cm^{-1}	118	0.881	0.995	0.991	1.37E+4	3.61E+4	9.01	0.849	0.999	0.999	2.56E+4	8.22E+4
1586 cm^{-1}	93	0.508	0.982	0.964	2.16E+4	6.91E+4	15.5	1.17	0.999	0.998	6.17E+4	1.41E+5
1618 cm^{-1}	196	0.471	0.990	0.981	3.97E+4	9.49E+4	28.5	0.618	0.998	0.995	6.88E+4	1.99E+5

SERS Spectra for Entire Target Concentration. The SERS spectra were obtained for the entire concentration range analysed.

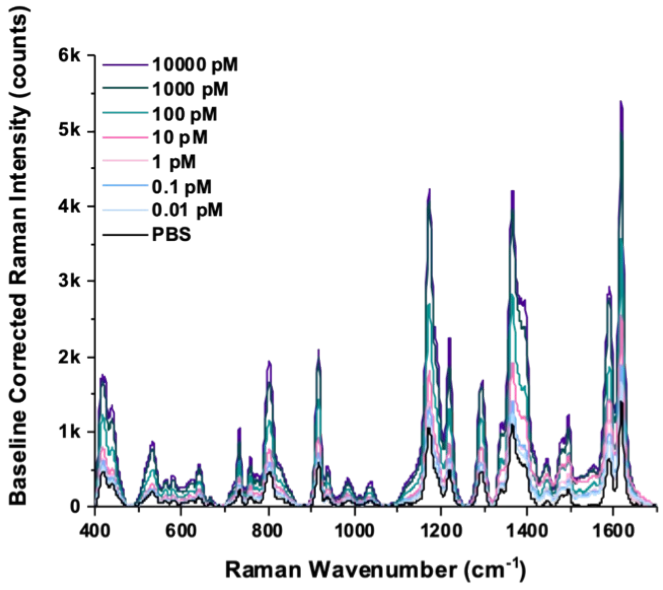

Figure S4. The SERS plot of the entire concentration range of miRNA-17 target tested along with the assay without target (PBS).

Statistical Analysis of SERS Data Using Paired t-Tests. A paired t-test was used to identify the quantifiable range of the detection assay for miRNA-17. Every possible combination was tested to determine the degree of signal separation between the various target miRNA-17 concentrations. A full summary of the results is provided in Table S4. The table has been formatted with the t values located to the right of the divider and the probability to the left.

Table S4. Results of paired t-Test between consecutive target analyte concentrations

	PBS	10 nM	1 nM	100 pM	10 pM	1 pM	100 fM	10 fM
PBS		3.6E-4	0.0035	1.8E-4	7.7E-4	0.014	0.0023	0.0010
10 nM	52.8		0.62	8.5E-4	8.7E-4	4.2E-4	7.7E-5	2.3E-4
1 nM	16.9	0.579		0.027	0.014	0.012	0.0084	0.0074
100 pM	74.5	34.4	6.00		0.0023	0.0046	1.4E-4	2.0E-4
10 pM	36.1	33.9	8.33	21.0		0.031	0.0043	0.0021
1 pM	8.30	49.0	9.22	14.7	5.54		0.86	0.60
100 fM	20.8	114	10.8	85.1	15.3	0.196		0.14
10 fM	31.2	66.2	11.5	72.0	21.6	0.610	2.34	

Table S5. Paired t-Test of signal intensity at different miRNA-17 Target concentrations using the peak at 1618 cm^{-1}

	STATISTICAL ANALYSIS USING PAIRED t-TEST					
	DETECTION ASSAY IN PBS			*DETECTION ASSAY IN 20% (v/v) BOVINE SERUM*		
	t Value	**p Value**	**SIGNIFICANT**	**t Value**	**p Value**	**SIGNIFICANT**
PBS vs. 10 nM	52.8	0.00036	YES	19.4	0.0027	YES
10 nM vs. 1 nM	***-0.579***	***0.62***	***NO***	***23.5***	***0.144***	***NO***
1 nM vs. 100 pM	6.00	0.027	YES	-62.4	0.00026	YES
100 pM vs. 10 pM	21.0	0.023	YES	13.3	0.00056	YES
10 pM vs. 1 pM	5.54	0.031	YES	-12.7	0.0061	YES
1 pM vs. 100 fM	***0.196***	***0.86***	***NO***	***1.80***	***0.215***	***NO***
100 fM vs. 10 fM	***2.34***	***0.14***	***NO***	***12.1***	***0.052***	***NO***
10 fM vs. PBS	31.2	0.0010	YES	-50.6	0.00039	YES

ANOVA Regression Analysis of the Characteristic Raman Peaks. A semi-log plot of the peak area was obtained for each of the five characteristic peaks of MGITC. The data included in the plots corresponded to the quantifiable range of the miRNA-17 detection assay. The results of the regression analysis at each of the five characteristic peaks is summarized in Table S6.

Table S6. Summary of fit parameters from the linear regression analysis of the assay in PBS and diluted bovine serum

	LINEAR REGRESSION ANALYSIS									
	DETECTION ASSAY IN PBS					***DETECTION ASSAY IN 20% (v/v) BOVINE SERUM***				
PEAK (cm^{-1})	**1180**	**1220**	**1290**	**1618**	**1620**	**1180**	**1220**	**1290**	**1618**	**1620**
SLOPE	2.66E4	6.84E3	6.70E3	1.13E4	1.48E4	5.29E4	8.38E3	1.52E4	2.64E4	3.69E4
INTERCEPT	3.60E5	9.33E4	9.24E4	1.60E5	2.16E5	7.42E5	1.17E4	2.22E4	3.83E5	5.20E5
ADJ R-SQUARED	0.884	0.915	0.913	0.911	0.925	0.910	0.952	0.917	0.916	0.977
PEARSON'S R	0.961	0.971	0.970	0.970	0.975	0.969	0.984	0.972	0.972	0.992
R-SQUARE (COD)	0.923	0.943	0.942	0.941	0.950	0.940	0.952	0.945	0.944	0.985
F VALUE	23.9	33.2	32.3	31.8	38.0	31.2	129	33.8	34.1	60.1
p VALUE	0.0393	0.0289	0.0296	0.0300	0.0253	0.0306	0.00768	0.0284	0.0281	0.0162

Statistical Significance Analysis of Assay Variables. A One-Way ANOVA was used to determine the statistical significance of Raman peak location and target miRNA-17 concentration with regards to SERS signal intensity. A result summary for the ANOVA analysis comparing Raman peak location with SERS signal intensity is provided in Table S6. The analysis showed that each Raman peak location examined significantly influenced signal intensity. Additionally, the data from the ANOVA test determining the statistical significance of the relationship between miRNA-17 concentration and SERS signal intensity is presented in Table S6. Based on the data, the concentration of miRNA-17 is statistically significant to the signal intensity.

Table S7. Summary of results of a One-Way ANOVA analysis of the SERS signal intensity to determine the significance of Raman peak location

	COMPARISON OF SERS SIGNAL INTENSITY VERSUS MIRNA-17 TARGET CONCENTRATION								
	DETECTION ASSAY IN PBS					***DETECTION ASSAY IN 20% (v/v) BOVINE SERUM***			
PEAK LOCATION	**DF**	**SUM OF SQUARES**	**MEAN SQUARE**	**F**	**P-VALUE**	**SUM OF SQUARES**	**MEAN SQUARE**	**F**	**P-VALUE**
1177 cm^{-1}	3	1.15E10	3.82E9	88.1	1.81E-6	3.36E10	1.12E10	146	2.52E-7
1220 cm^{-1}	3	7.43E8	2.48E8	21.5	3.50E-4	1.07E9	3.57E8	219	5.11E-8
1290 cm^{-1}	3	7.15E8	2.38E8	65.2	5.78E-6	4.37E9	1.46E9	178	1.16E-7
1586 cm^{-1}	3	2.03E9	6.77E8	60.8	7.53E-6	1.10E10	3.67E9	130	4.03E-7
1618 cm^{-1}	3	2.66E9	8.88E8	55.0	1.11E-5	2.03E10	6.75E9	113	6.79E-7

Table S8. Summary of results of a One-Way ANOVA analysis of the SERS signal intensity to determine the significance of the target concentration

	COMPARISON OF SERS SIGNAL INTENSITY WITH RAMAN PEAK LOCATION								
	DETECTION ASSAY IN PBS					***DETECTION ASSAY IN 20% (v/v) BOVINE SERUM***			
TARGET CONCENTRATION	**DF**	**SUM OF SQUARES**	**MEAN SQUARE**	**F**	**P-VALUE**	**SUM OF SQUARES**	**MEAN SQUARE**	**F**	**P-VALUE**
1 nM	4	1.91E10	4.78E9	78.1	1.68E-7	7.45E10	1.86E10	333	1.37E-10
100 pM	4	8.94E9	2.23E9	831	1.45E-12	5.99E10	1.50E10	301	2.24E-10
10 pM	4	4.37E9	1.09E9	859	1.23E-12	3.70E10	9.24E9	1350	1.27E-13
1 pM	4	3.21	8.02E8	272	3.74E-10	1.38E10	3.45E9	128	1.53E-8

Statistical Analysis of Non-Complimentary Control Strands Using Paired t-Tests. A paired t-test to determine the degree of signal separation between the non-complementary oligonucleotide strands with the assay in PBS.

Table S9. Paired t-Test of the signal intensity of the forward and reverse non-complimentary strands of the negative control RNA

	FORWARD STRAND		REVERSE STRAND	
	t Value	p Value	t Value	p Value
miRNA 34-3p vs. PBS	0.904	0.42	-1.18	0.30
miRNA 126-3p vs. PBS	0.629	0.56	-1.56	0.19
miRNA 155-3p vs. PBS	2.38	0.076	-0.792	0.47
miRNA 210-3p vs. PBS	-1.06	0.35	-0.624	0.57
RNA U6 vs. PBS	-0.169	0.87	-0.670	0.54

Evaluation of the Assay in a Complex Biological Medium. The assay was tested in diluted bovine serum to determine its sensing capacity in a complex sample.

Table S10. Vibrational peak assignment of the prominent Raman peaks of diluted bovine serum

PEAK (cm^{-1})	VIBRATIONAL MODE	MAJOR ASSIGNMENT
872	ν(C-C)	Tyrosine, Proline, Hydroxyproline
959	δ_{CH3} (Deformed) Hydroxyapatite, carotenoid, cholesterol	Lipid, Protein
1004	ν_s(C-C)	Phenylalanine (Protein)
1157	ν(C-C) and ν(C-N)	β -Carotenoids (Protein)
1190	δ(C-H)	Tryptophan (Protein), Phenylalanine (Protein)
1210	Tryptophan and Phenylalanine	Tryptophan (Protein), Phenylalanine (Protein)
1269	ν(C-N), δ(N-H)	Amide III (Protein), Collagen
1351	CH_3CH_2 Wagging	Collagen (Protein)
1445	δ(CH_2)	Collagen (Protein), Phospholipids (Lipids)
1521	ν(C=C)	β -Carotenoids (Protein)
1593	δ(C=C)	Phenylalanine (Protein), Hemoglobin
1691	Amide 1, α-helix ν(C=O)	Protein, Phospholipids

Analysis of Bovine Serum. The sample solution of bovine serum was tested to determine if the target analyte, miRNA-17, was initially present in the sample prior to doping. Real-time reverse transcription PCR is commonly used for detecting miRNA in solution. Thus, PCR analysis was obtained for the bovine serum solution and the results were compared to a control solution (water).

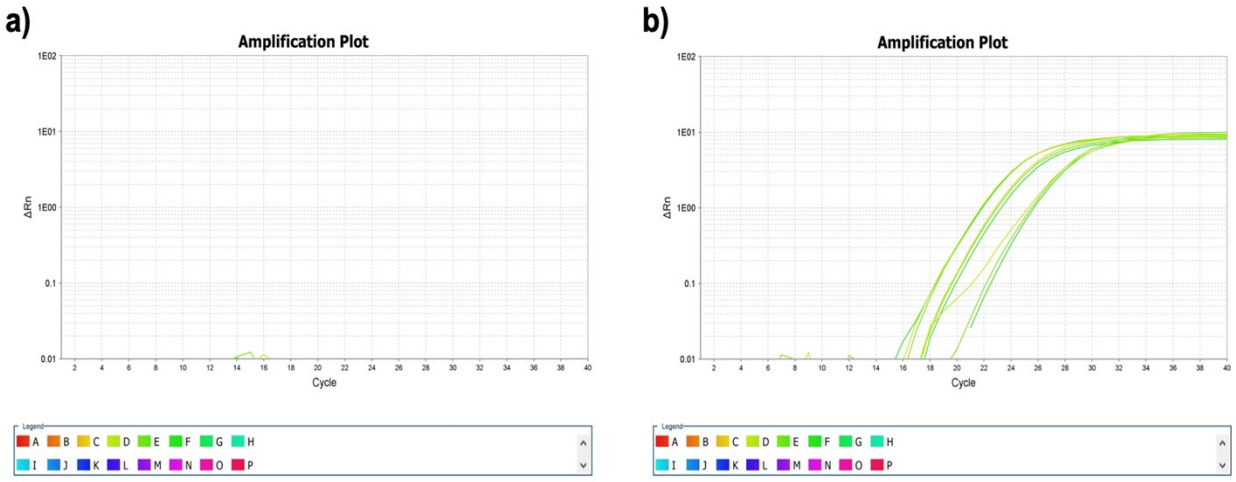

Figure S5. The resultant amplification plots obtained from the real-time reverse transcription PCR (qRT-PCR) for miRNA-17 in a) water and b) bovine serum solution.

SERS Spectra of the Assay in Bovine Serum. The resultant SERS spectra obtained when testing the miRNA-17 detection assay in the complex biological medium of bovine serum.

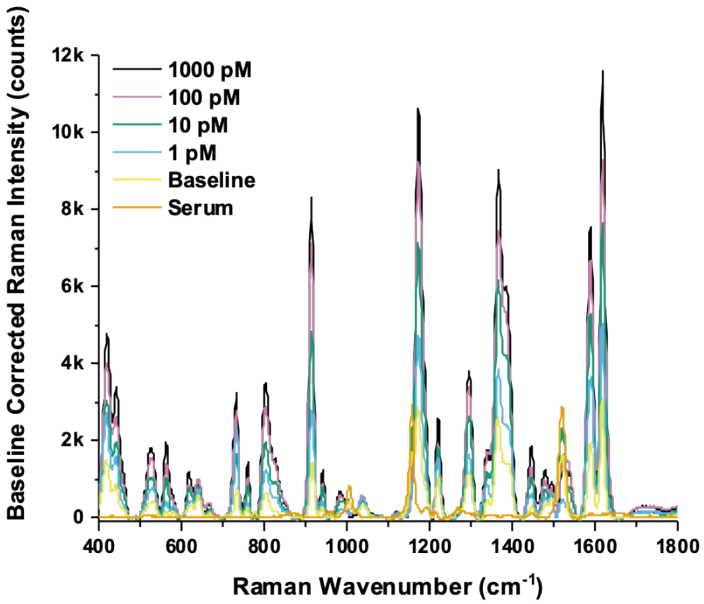

Figure S6. Raman spectra of the diluted bovine serum and the normalized SERS signal of the miRNA-17 detection assay tested in diluted bovine serum

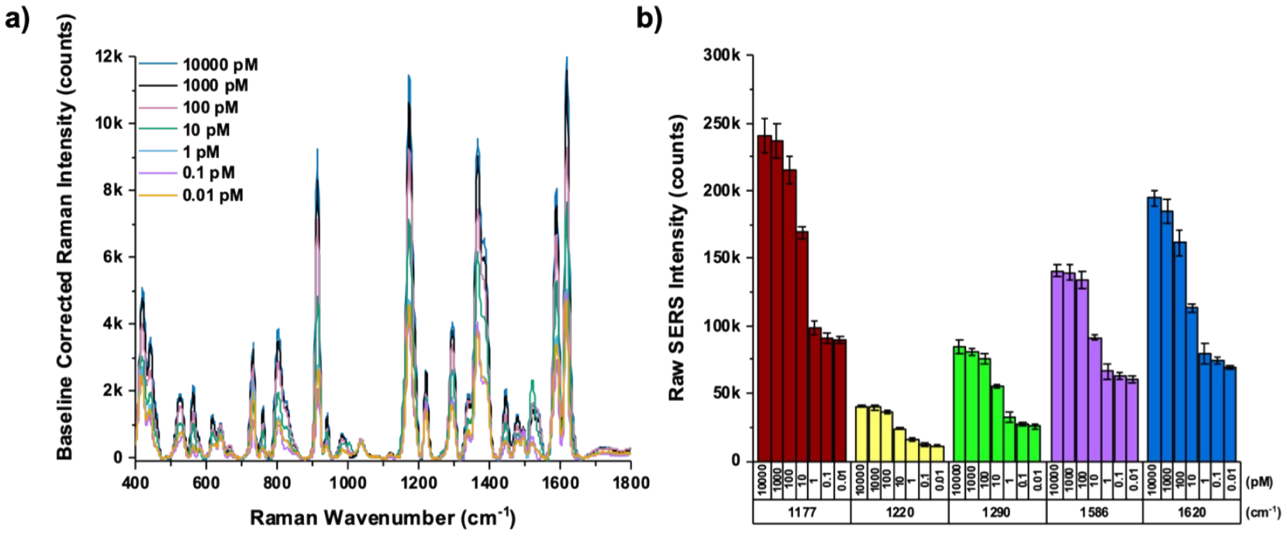

Figure S7. The (a) raw SERS spectra for the entire target range and (b) integrated peak area for the characteristic peak of MGITC centred at 1618 cm^{-1} for different concentrations of miRNA-17 suspended in diluted bovine serum.

Analysis of Assay in Bovine Serum. Analysis of the data collected from the SERS spectra obtained when testing the miRNA-17 detection assay in the complex biological medium of bovine serum.

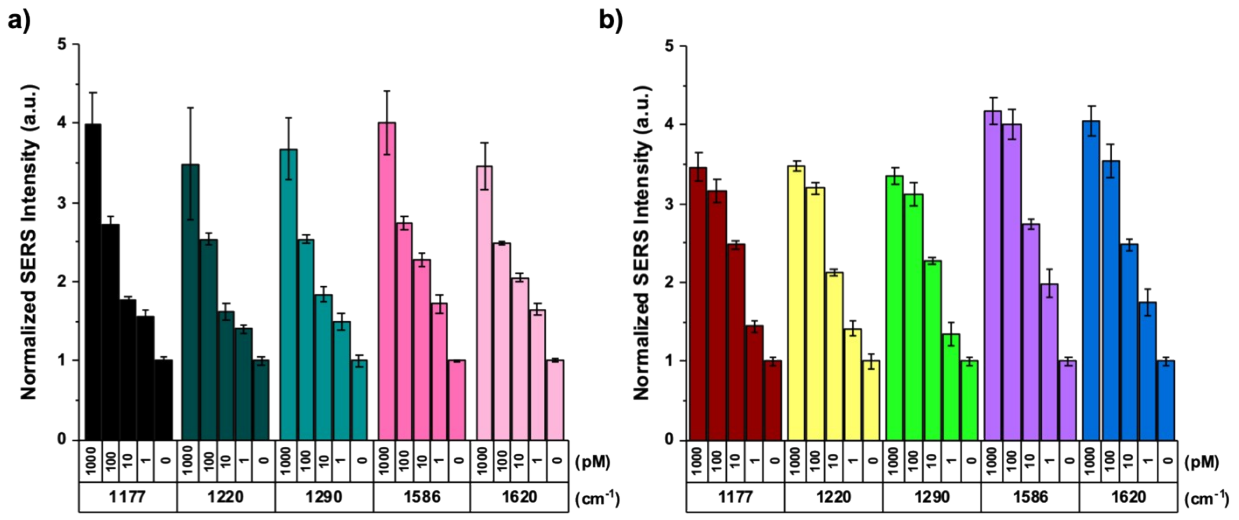

Figure S8. The normalized peak area of the for the characteristic peaks of MGITC at different concentrations of miRNA-17 for the assay in (a) PBS and(b) diluted bovine serum. The results were normalized with respect to the control spectra which is referred to in the figure as results obtained from the assay with 0 pM of target.