# FEATURE HIGHLIGHT: *Precise Kinetic Characterization in Serum*

Details taken from Application Note: **Reproducible Kinetic Characterization of Small Molecule Compounds in Serum with Agile R100.** Read the full App Note.


## Agile™ R100

### *Serum clearly* for the first time.

Agile R100 is the label-free personal assay system that **lets you measure in serum with little to no assay adjustment from buffer protocols.** The biosensor leverages a breakthrough electrical technique called Field Effect Biosensing to easily detect in complex matrices without the interference that can affect optical sensing platforms. This means you can incorporate serum effects earlier in your drug discovery process, without time-consuming and costly assay development and optimization.

### PAPER SUMMARY

- In this application note, we show buffer and serum interactions for 3 target and small molecule binding pairs: a chemokine binding G-protein coupled receptor (CR GPCR) binding with small molecule Compound A, the cytokine tumor necrosis factor alpha (TNFα) protein binding with small molecule SPD304, and a DNA aptamer against colistin (i.e. colistin aptamer) binding with the antibiotic drug colistin. **No assay adjustment is made in the transition from buffer to serum.**

- **All kinetic constants between buffer and serum for the 3 binding interactions are within a factor of 1.9**, displaying a high level of precision among multiple replicates and measurements.


### OVERVIEW

Assays often need to be greatly modified when studying a compound in serum versus buffer. Frequently, different tools are used, necessitating the development of entirely new experimental conditions. Even when the same platform is utilized, limited ability to measure in complex matrices forces additional assay re-optimization, expending precious time and resources.

Obtaining small molecule kinetic information in serum earlier in the drug discovery process provides a significant edge in determining the likelihood of success of a drug. Agile R100 uses Field Effect Biosensing (FEB) to detect small molecule interactions in serum without the interference and noise common on other platforms. **Minimal to no protocol changes are needed to transition from buffer to serum which drastically reduces development time and cost, bringing early kinetic characterization in serum within the reach of any lab.**

## MATERIALS AND METHODS

### Biosensor Chip Immobilization

Three targets were immobilized to biosensor chips using protocols from Agile Plus software. Specifically, 300 nM His-tagged CR GPCR was added to NTA biosensor chips, 10.2 nM TNFα cytokine was added to NHS biosensor chips, and 100 nM colistin aptamer was added to bare biosensor chips. The immobilization buffer was 1X PBS pH 7.4, 1% DDM, 1% CHS for CR, 1X PBS pH 7.4 for TNFα, and 1X PBS pH 7.4, 1 mM $MgCl_2$ for colistin aptamer.

To immobilize CR, the NTA biosensor chips were first activated with $NiCl_2$, followed by target addition for 15 minutes. For TNFα, target was added to the NHS biosensor chips for 15 minutes, followed by blocking and quenching with Quench 1 and Quench 2 for 15 minutes each. The colistin aptamer with an attached pyrene group was added directly to bare biosensor chips for 15 minutes, and Quench 1 was added for 15 minutes to serve as a block.

### Biosensor Chip Measurement

The small molecules interacting with CR, TNFα, and colistin aptamer were Compound A, SPD304, and colistin, respectively. For the buffer experiments (n = 3), the assay buffer was 1X PBS pH 7.4, 1% DDM, 1% CHS, 0.2% DMSO for Compound A, 1X PBS pH 7.4 for SPD304, and 1X PBS pH 7.4, 1 mM $MgCl_2$ for colistin. For the serum experiments (n = 4), normal rat serum was added to each assay buffer to a final concentration of 1%.

Biosensor chips were calibrated in fresh assay buffer, followed by the addition of analyte in the respective assay buffer for 5 minutes. The CR biosensor chips were exposed to 100 µM Compound A, the TNFα biosensor chips were exposed to 5 µM SPD304, and the colistin aptamer biosensor chips were exposed to 10 µM colistin. Fresh assay buffer was then added to initiate dissociation for 5 minutes. The $k_{on}$, $k_{off}$, and $K_D$ were calculated in Agile Plus software and were compared for buffer and serum.

## RESULTS AND DISCUSSION

For each interaction, reproducible kinetic binding results were calculated with n = 3 for each interaction in buffer and n = 4 for each interaction in serum. The average $k_{on}$, $k_{off}$, and $K_D$ for each measurement in buffer and serum are summarized in the tables on the right.

For the CR GPCR and Compound A interaction, the kinetic binding data agree within a factor of 1.5 between buffer and serum. For the TNFα cytokine and SPD304 interaction, the kinetic binding data agree **within a factor of 1.9** between buffer and serum, and for the colistin aptamer and colistin interaction, the data agree **within a factor of 1.7, illustrating Agile R100's ability to measure interactions in buffer and serum with high precision.**

Accurate characterization of small molecule and target interactions is crucial for successful drug discovery. By eliminating compounds that do not perform well in serum earlier in the process, researchers can make more confident decisions while reducing time and cost. Agile R100 reproducibly characterizes small molecules in serum using FEB, an electrical label-free technique that is not subject to the serum interference that affects other assay tools. In this application note, Agile R100 precisely measures the binding kinetics of 3 low molecular weight compounds interacting with their targets in both buffer and serum with no assay modification, and the kinetic binding data compared between buffer and serum are within a factor of 1.9 for all 3 binding pairs. This demonstrates the ability to measure in serum with little to no assay adjustment from buffer, letting researchers easily gain information-rich data without extensive time and expense.

**CR and Compound A**

| | $k_{on}$ ($M^{-1}s^{-1}$) | $k_{off}$ ($ks^{-1}$) | $K_D$ (µM) |
|---|---|---|---|
| **Buffer** | 155 ± 54.9 | 5.04 ± 1.79 | 38.4 ± 18.8 |
| **Serum** | 134 ± 56.0 | 7.81 ± 2.95 | 59.1 ± 6.25 |

**TNFα and SPD304**

| | $k_{on}$ ($M^{-1}s^{-1}$) | $k_{off}$ ($ks^{-1}$) | $K_D$ (µM) |
|---|---|---|---|
| **Buffer** | 2500 ± 198 | 8.02 ± 1.27 | 3.19 ± 0.278 |
| **Serum** | 2520 ± 491 | 14.3 ± 1.23 | 5.94 ± 1.48 |

**Colistin aptamer and colistin sulfate**

| | $k_{on}$ ($M^{-1}s^{-1}$) | $k_{off}$ ($ks^{-1}$) | $K_D$ (µM) |
|---|---|---|---|
| **Buffer** | 797 ± 313 | 5.00 ± 1.04 | 6.68 ± 1.11 |
| **Serum** | 471 ± 144 | 3.53 ± 0.934 | 7.58 ± 0.853 |

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We're here to help you achieve the information-rich data you need when you need it. Contact us to learn more!

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