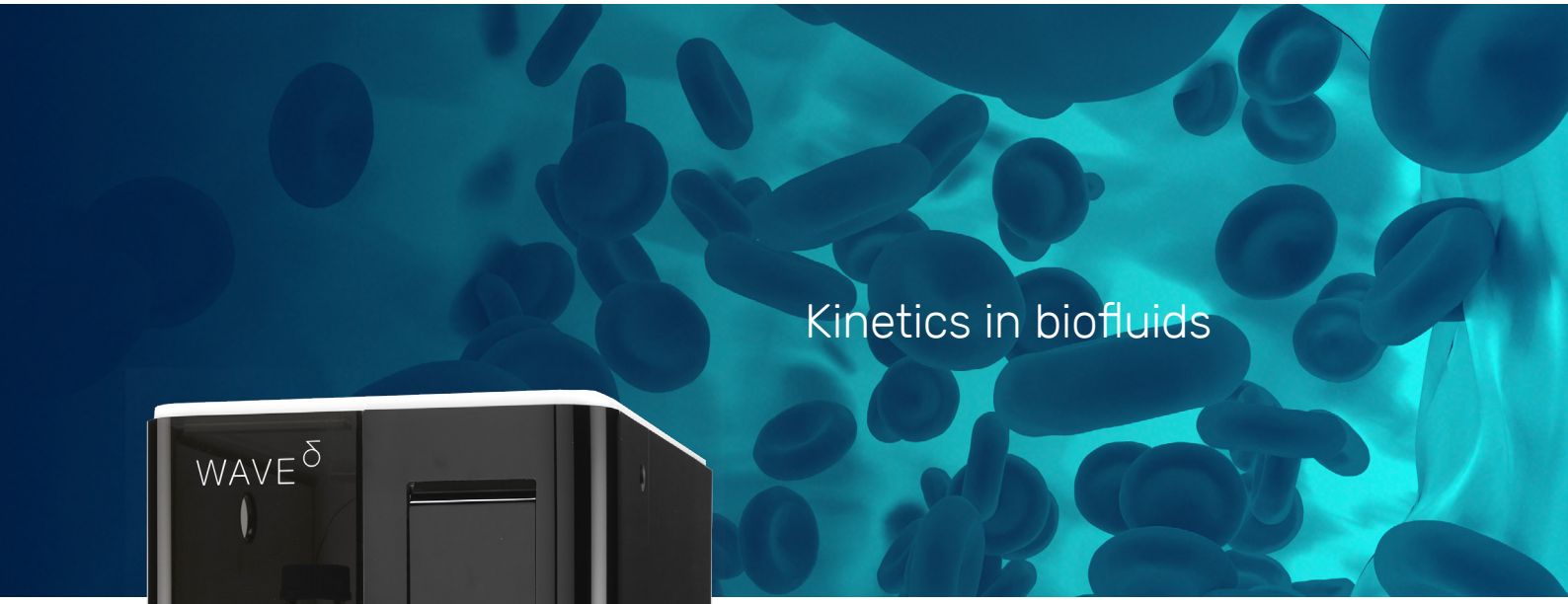
creoptix sensors

TechNote 10

Affinity and kinetics of antibodies in serum

Creoptix® WAVEsystem



In this TechNote we show how the WAVE system can be used to accurately determine kinetics in serum. Thanks to a no-clog microfluidic design, kinetic studies can be performed under conditions that mimic the native environment as closely as possible, enabling the translation of these results to the clinic

Summary

Label-free analysis of molecular interactions in complex biofluids such as serum provides valuable information to guide therapeutic antibody studies. However, these matrices often put surface-based biosensors under considerable stress as various components cause the microfluidics to clog. This can result in lengthy program delays while essential maintenance is performed.

Employing innovative, disposable microfluidics, the Creoptix® WAVEsystem is highly resistant to clogging. This makes kinetic analysis in physiologically relevant matrices possible, providing superior insight compared to traditional Surface Plasmon Resonance (SPR) technologies.

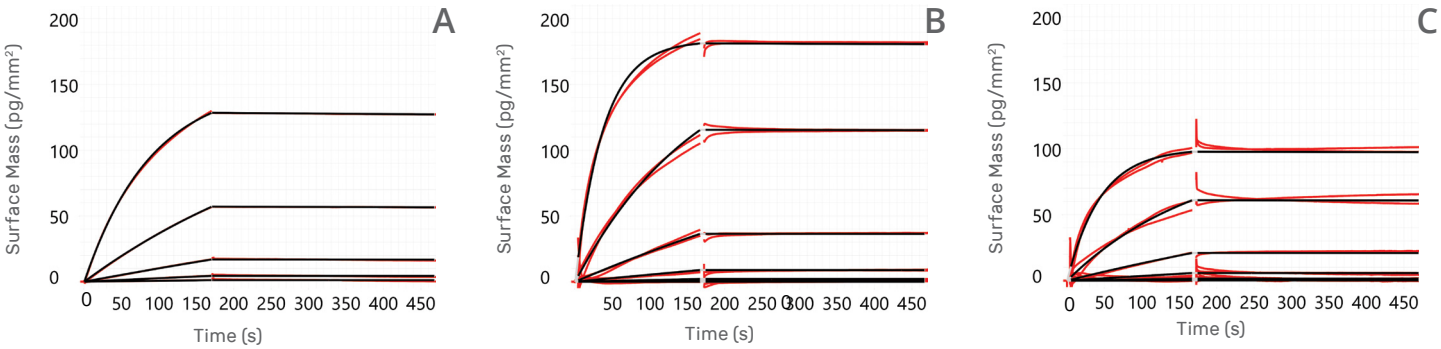
By measuring the interaction between HER2 and trastuzumab in matrices containing different concentrations of serum, we show that the Creoptix WAVEsystem, with its clog-free microfluidics, delivers robust kinetic analysis of antibody binding in biofluids.

On a 4PCP WAVEchip, HER2-Fc fusion (Sino Biological, CN) was immobilized on a channel at 975pg/mm2 via amine coupling, followed by BSA passivation (Roche, CH) on all channels to reach 4200pg/mm^2 of total protein per channel. Running buffer (RB) used for all experiements was (HBS-EP+ supplemented with 0.5% BSA). Trastuzumab (Absolute Antibody, UK) was injected at 150 ul/min for 170s, followed by a 300s dissociation step. for the 0% serum condition, Trastuzumab was prepared in 100% RB and diluted from 20 nM (4-fold dilution series).
for the 50% serum condition, Trastuzumab was prepared from 100 nM in 1:1 (v/v) serum:RB. for the 90% serum condition, Trastuzumab was prepared from 100 nM in 9:1 (v/v) serum:RB. Regeneration was achieved each time by injecting a pulse of 50mM NaOH, 1M NaCl. Measurements were adjusted using DMSO calibration, double referencing and bulk correction during evaluation.

Table 1: Kinetic data for the interaction between HER2 and trastuzumab at different serum concentrations

	Kinetic data	R_{max} (pg/mm^2)	k_{on} ($M^{-1}.s^{-1}$)	k_{off} (s^{-1})	K_D (pM)
A	0% Serum	151.4	$5.58x10^5$	$3.19x10^{-5}$	57.1
B	50% Serum	183.5	$2.68x10^5$	$9.1x10^{-6}$	34
C	90% Serum	100.1	$2.25x10^5$	$9.49x10^{-6}$	42.1

Figure 1: Sensorgrams of the interaction between HER2 and trastuzumab in different matrices



1 pg/mm^2 = 1 RU

3013 TN 10/2020 2

Keeping Kinetics Real

Creoptix AG | Zugerstrasse 76 | 8820 Wädenswil | Switzerland (HQ)
Creoptix Inc | c/o The Brighton Company | 111 South Bedford Street, Ste. 108 | MA-01803 Burlington | USA
www.creoptix.com | +41 44 533 26 60