

# SPR 분석서비스 결과

아이클루바이오

## ❖ 분석 내용 및 샘플 정보

Experiment	Ligand	Analyte
단백질과 화합물의 결합 확인 및 kinetic evaluation	Carbonic Anhydrase II	Furosemide

## ❖ 분석 정보

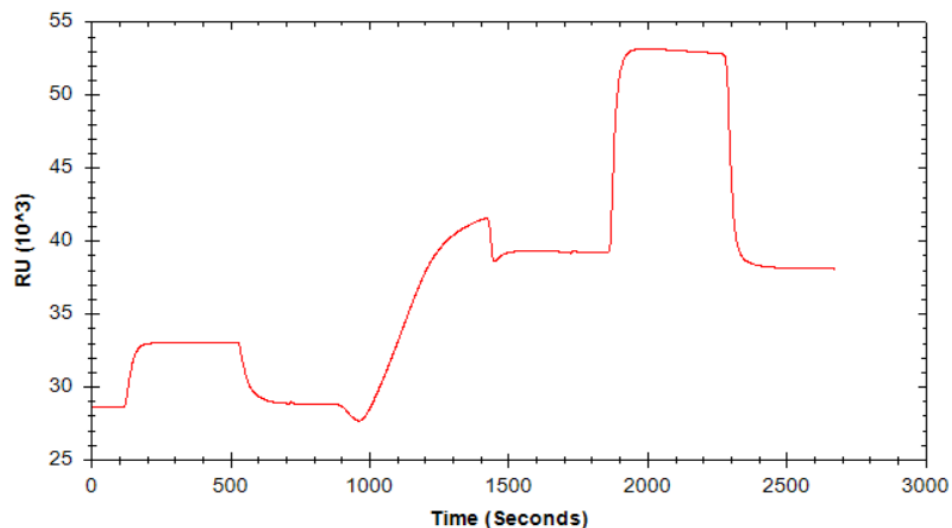
- Date: 2023-07-24 ~ 2023-07-25
- Instrument: iMSPR-ProX

## ❖ Immobilization

- Amine coupling
- Sensor chip: HC1000M
- Running buffer: 10 mM Phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4
- Flow rate: 10  $\mu$ l/min

Immobilization	
<b>Ch 1 (Ligand)</b>	<b>Activation</b> 200 mM EDC+100 mM NHS, 7-min injection
	<b>Ligand injection</b> 50 $\mu$ g/ml Carbonic Anhydrase II in 5 mM acetate buffer (pH 5.0), Inject time: 9-min
	<b>Blocking</b> 1 M Ethanolamine*HCl, 7-min injection
<b>Ch 2 (blank)</b>	<b>Activation</b> 200 mM EDC+100 mM NHS, 7-min injection
	<b>Blocking</b> 1 M Ethanolamine*HCl, 7-min injection

## ❖ Immobilization Results



Immobilization level: 9257.8 RU

## ❖ Binding Analysis

- Running buffer: 10 mM Phosphate, 137 mM NaCl, 2.7 mM KCl, 1% DMSO, pH 7.4
- Flow rate: 50 µl/min
- Analyte concentration: 10 µM

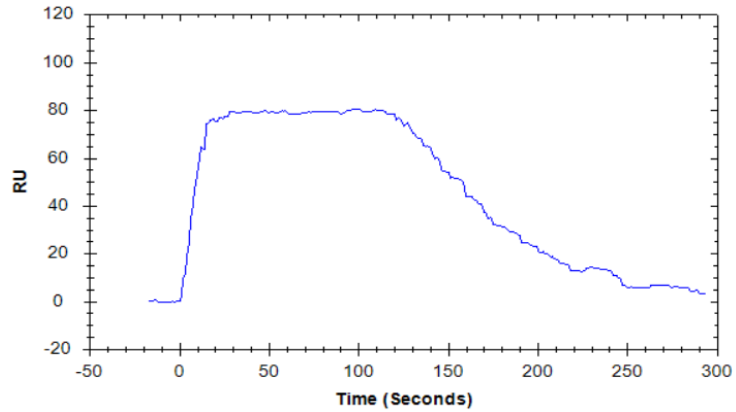
## ❖ Regeneration

- Remove the analyte
- Regeneration solution: 3 M NaCl
- Flow rate: 50 µl/min

Binding Analysis	
Ch 1-2	<b>Association time</b> 2 min
	<b>Dissociation time</b> 2.5 min

Regeneration	
Ch 1-2	<b>Injection time</b> 1 min
	<b>Stabilization time</b> 5 min

## ❖ Furosemide\_10 $\mu$ M



→ Kinetic 분석 기준 충족

## ❖ 분석 기준

**Affinity 분석 기준**

: RU(association) > 10RU

**Kinetic 분석 기준**

: RU(association) > 10RU

RU(dissociation) > 5RU

## ❖ Binding Analysis

- Running buffer: 10 mM Phosphate, 137 mM NaCl, 2.7 mM KCl, 1% DMSO, pH 7.4
- Flow rate: 50 µl/min
- Analyte concentration: 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10, 0 µM

## ❖ Regeneration

- Remove the analyte
- Regeneration solution: 1 M NaCl
- Flow rate: 50 µl/min

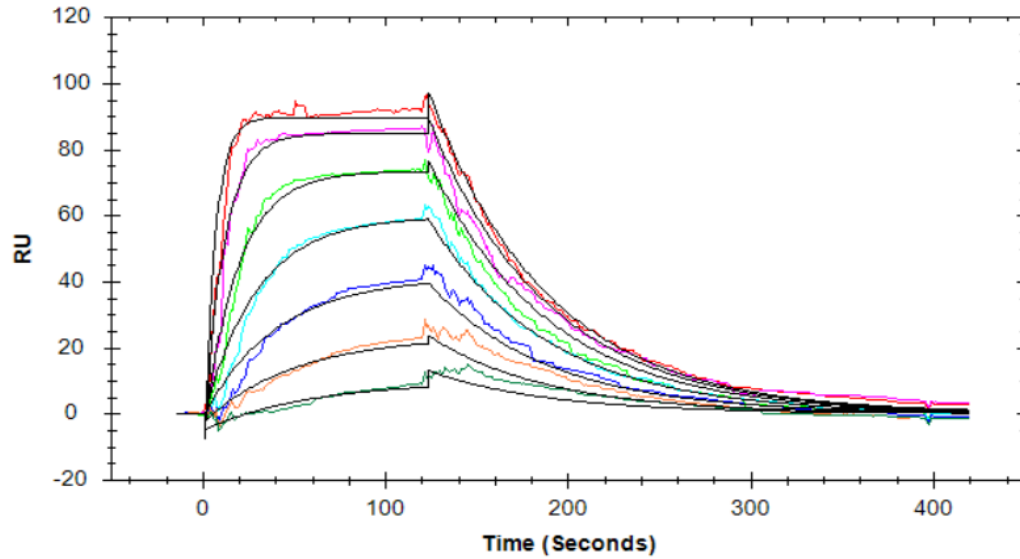
## ❖ Evaluation

- Evaluation S/W: TraceDrawer

Binding Analysis	
Ch 1-2	<b>Association time</b> 2 min
	<b>Dissociation time</b> 6 min

Regeneration	
Ch 1-2	<b>Injection time</b> 1 min
	<b>Stabilization time</b> 5 min

## ❖ Furosemide



	$k_a$ (1/Ms)	$k_d$ (1/s)	$R_{max}$ (RU)	RI (RU)	$K_A$ (1/M)	$K_D$ (M)	Chi <sup>2</sup>
	1.54E+04	1.52E-02	106.61		1.01E+06	9.87E-07	6.72
0.156 $\mu$ M				-4.87			
0.313 $\mu$ M				-22.3			
0.625 $\mu$ M				0.07			
1.25 $\mu$ M				0.11			
2.5 $\mu$ M				-3.15			
5 $\mu$ M				-4.26			
10 $\mu$ M				-7.41			