Quality test of –EPO biopharmaceutical using iMSPR-ProX & COOH chip

EPO is a glycoprotein hormone that induces red blood cell production and is a product that is being actively developed as a biobetter. The experiment using SPR can improve the precision and ethical aspects of the existing EPO potency assay using rats. In this application experiment, the affinity between EPO and EPO-R using iMSPR-ProX is evaluated, and this can present quality evaluation standards.

Materials

- Instrument: iMSPR-ProX
- Sensor chip: COOH-Au chip, Carboxyl Dextran-Au chip, 50nm (Cat. No DCCH1050)
- Immobilization Reagent: Amine coupling kit (ACK50)
- Immobilization buffer: Acetate buffer pH4.0 (AB40)
- Running buffer: 1xHBST (HB50)
- Regeneration buffer: 10 mM glycine pH 2.5
- Ligand: EPO-R
- Analyte: EPO

Procedure

Ligand Immobilization

① Baseline: 1xHBST is flowed into both channels (ligand channel, reference channel) at a flow rate of 30 µl/min for more than 5 minutes to establish a stable baseline.

② Inject a 1:1 mixture of NHS/EDC into both channels at a flow rate of 30 µl/min for 5 minutes followed by 5 minutes of washing in 1xHBST.

③ Inject 10 µg/ml 300ul of EPO-R (in Acetate buffer pH4.0) only into the ligand channel at a flow rate of 10 µl/min for 15 minutes followed by 5 minutes of washing in 1xHBST.

④ Inject 200 µl of Quenching buffer into both channels at a flow rate of 30 µl/min for 2 minutes followed by 5 minutes of washing in 1xHBST.

⑤ Inject 200 µl of Glycine pH 1.5 into both channels at a flow rate of 30 µl/min for 2 minutes followed by 30 minutes or more of washing in 1xHBST to stabilize.

Analyte binding

① Prepare 200 µl EPO analyte dilutions into the running buffer (on the same day) to a concentration of 1.56, 3.13, 6.25, 12.5, 25, 50, 100, 0 nM.

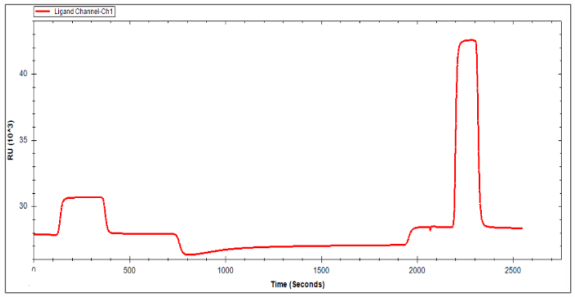
② Introduce 1.56 nM EPO at 30 µl/min for a 3 minutes association time followed by 1xHBST for a 6-minute dissociation time.

③ After binding, the surface was regenerated by injecting Glycine pH 2.5 solution at a flow rate of 10 µl/min for 30 seconds. And then washed with 1xHBST for 6 minutes to stabilize.

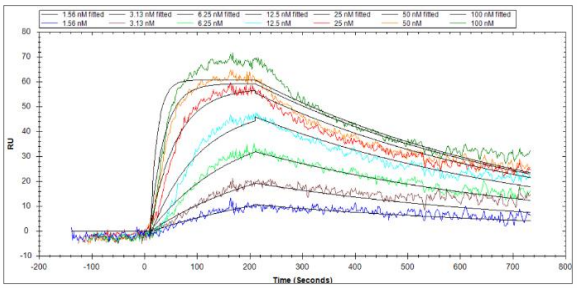
④ Repeat ②-③ for each concentration.

Results

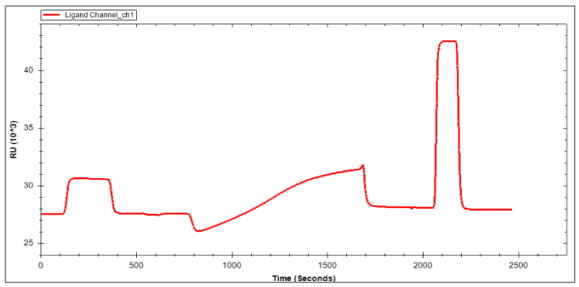
R1. Ligand Immobilization (COOH-Au chip)



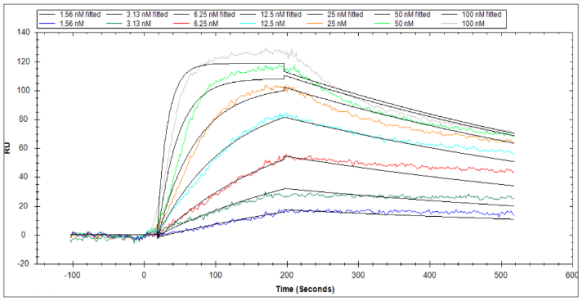
R2. Curve fitting: one to one binding model (COOH-Au chip)



R3. Ligand Immobilization (Carboxyl Dextran-Au chip)



R4. Curve fitting: one to one binding model (Carboxyl Dextran-Au chip)



Results summary

T1. Kinetic Evaluation (COOH-Au chip)

Contents	Value
Immobilization Level	1058 RU
R_{max}	62.24 RU
K_a (Association rate, 1/M*s)	7.26 x 10^5
K_d (Dissociation rate, 1/s)	1.82 x 10^{-3}
K_D (Affinity)	2.50 x10^{-9} M
Chi^2	10.07

T2. Kinetic Evaluation (Carboxyl Dextran-Au chip)

Contents	Value
Immobilization Level	830 RU
R_{max}	115.46 RU
K_a (Association rate, 1/M*s)	6.79 x 10^5
K_d (Dissociation rate, 1/s)	1.47 x 10^{-3}
K_D (Affinity)	2.17 x10^{-9} M
Chi^2	18.81