Description

The NRG1β: HER4 (ERBB4) Chemiluminescent Assay Kit is an ELISA-based assay designed to measure the binding between NRG1β (neuregulin-1β) and HER4 (human epidermal growth factor receptor 4, also known as ERBB4) for screening and profiling applications. The NRG1β: HER4 (ERBB4) Chemiluminescent Assay Kit comes with enough purified NRG1β (amino acids 2-246) and HER4 (amino acids 26-651) proteins, detection antibody, assay buffer, and detection reagent for 100 enzyme reactions.

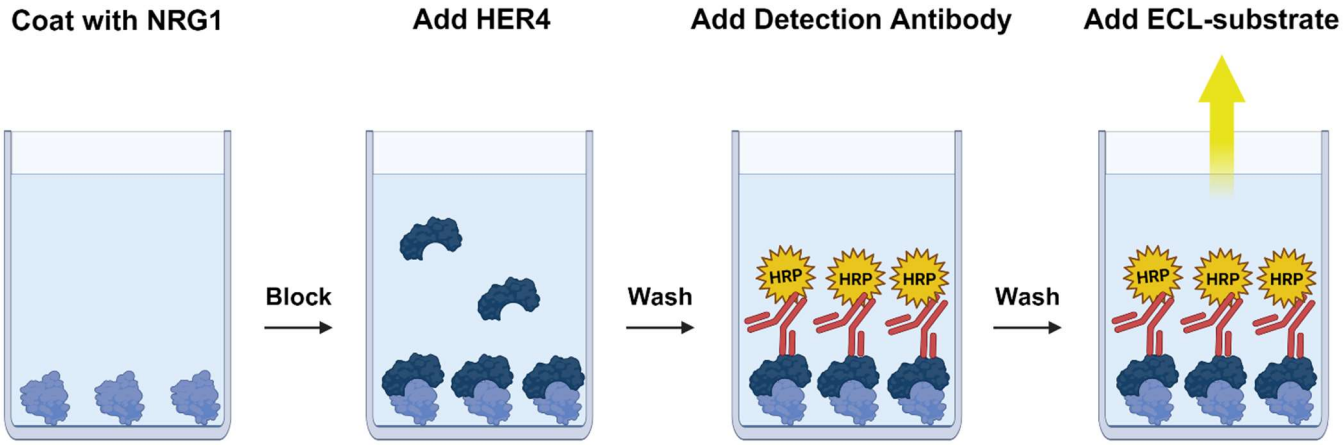

Figure 1. NRG1β: HER4 (ERBB4) Chemiluminescent Assay Kit schematic.
A 96-well plate is coated with NRG1β protein. After coating and blocking, HER4 is added in an optimized assay buffer. Unbound HER4 is washed away, and the plate is incubated with a detection antibody. Finally, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the efficacy of HER4 binding to NRG1β.

Background

HER4 (human epidermal growth factor receptor 4), also known as ErbB4 or receptor-tyrosine kinase 4, is a receptor tyrosine kinase protein of the EGFR family. It is required for the development of the heart, nervous system, and mammary gland, and can also be found in the postsynaptic side of neuromuscular junctions and epidermis. HER4 is activated by betacellulin and heparin binding EGF-like growth factor (HB-EGF) and is involved in mitogenesis and cell differentiation. HER4 undergoes a conformational change upon ligand binding and forms homodimers or heterodimers with other HER proteins to activate the MAPK (mitogen-activated protein kinases) and PI3K (phosphoinositide 3-kinase)/AKT pathways. Mutations in this protein can result in cancer but have also been linked to ALS (amyotrophic lateral sclerosis) type 19 and schizophrenia. The role of this protein as oncogene or suppressor is still not fully determined, potentially being dependent on the cancer type. Several HER4 inhibitors targeting the kinase domain of the protein have been developed and are undergoing clinical trials. Afatinib, a pan-HER inhibitor, has been approved for the treatment of NSCLC (non-small cell lung cancer), but may cause acute liver injury and death. Neuroregulins (NRG) are polypeptide growth factors, that bind to HER proteins, with NRG1 being the most studied of the four known proteins in this family. NRG1 specifically binds to HER3 and HER4. Binding of NRG1 to HER4 play an important role in cortical development, and cardiomyocyte proliferation and regeneration, by inducing dimerization to HER2 and activation of multiple signaling pathways. The high similarity between HER3 and HER4 make the development of specific antibodies challenging. Studies focusing on molecules targeting NRG1/HER4 will open new therapies for patients with neurodevelopment and cardiovascular disorders.

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Applications

Study complex formation and screen compounds and biologics that block the binding of NRG1β to HER4 for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	NRG1β 1 Protein, Human (HEK293)*	2.5 µg	-80°C
	HER4 (26-651-His)*	5 µg	-80°C
82620	5x PP-02 Buffer	4 ml	-80°C
	HRP-Labeled Detection Antibody 3	10 µl	-80°C
79743	Blocking Buffer 3	50 ml	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79837	96-well module plate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 1x PBS (Phosphate Buffer Saline) Buffer
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The NRG1β: HER4 (ERBB4) Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

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- We recommend using HER4 (ERBB4), Fc Fusion, Avi-Tag Recombinant (#102238) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1: Coat 96-well module

1. Thaw NRG1β on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute NRG1β protein to 0.5 ng/µl with PBS (50 µl/well).
3. Add 50 µl of diluted NRG1β to every well except "Blank" wells.
4. Add 50 µl of Blocking Buffer 3 to "Blank" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times using 200 µl of PBST Buffer per well.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Block the wells by adding 200 µl of Blocking Buffer 3 to every well.
9. Incubate at Room Temperature (RT) for at least 90 minutes.
10. Wash the plate three times using 200 µl of PBST Buffer per well.
11. Tap the plate onto clean paper towel to remove the liquid.

Step 2: Binding reaction

1. Prepare 1x Assay Buffer by diluting 5x PP-02 Assay Buffer 5-fold with distilled water.
2. Add 20 µl of 1x Assay Buffer to every well.
3. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   3.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using 1x Assay Buffer.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   3.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x Assay Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

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Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

4. Add 5 µl of Test Inhibitor to each well labeled as "Test Inhibitor".
5. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
6. Thaw HER4 on ice. Briefly spin the tube containing the protein to recover its full content.
7. Dilute HER4 to 2 ng/µl with 1x Assay Buffer (25 µl/well).
8. Add 25 µl of diluted HER4 to all wells.
9. Incubate at RT for 1 hour.

	Blank (non-coated wells)	**Positive Control**	**Test Inhibitor**
1x Assay Buffer	20 µl	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted HER4 (2 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

10. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.

Step 3: Detection

1. Dilute 1000-fold the HRP-labeled Detection Antibody 3 with Blocking Buffer 3 (50 µl/well).
2. Add 50 µl of diluted HRP-labeled Detection Antibody 3 to every well.
3. Incubate for 60 minutes at RT.
4. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.
5. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).
6. Add 100 µl of mix to every well.
7. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

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8. The "Blank" value should be subtracted from all other values.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

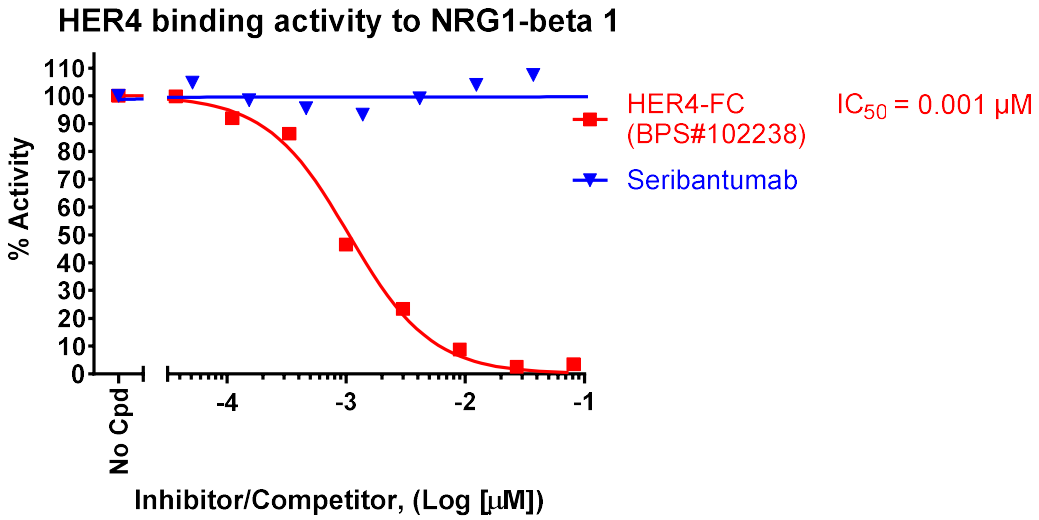

Figure 2: Inhibition of NRG1β: HER4 binding by the HER3 specific Antibody Seribantumab and HER4 (ERBB4), Fc Fusion, Avi-Tag Recombinant.
HER4 was incubated with increasing concentrations of Seribantumab (#82613) or HER4 (ERBB4), Fc Fusion, Avi-Tag Recombinant (#102238) in an NRG1β-coated plate. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding in which the condition without competitor is set to 100%.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

El-Gamal M., *et al.*, 2021 *Molecules* 26(23):7376.
Shi L. and Bergson C., 2020 *Translational Psychiatry* 10: 190.
Shan L., *et al.*, 2020 *Scientific Reporters* 10: 17257.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Chemi-Verse™ HER4 Kinase Assay Kit	82553	96 reactions
HER3 CHO Cell Line	82299	2 vials
HER3 (E928G), GST-Tag Recombinant	101645	20 µg/50 µg
HER3, GST-Tag Recombinant	101646	20 µg/50 µg
NRG1β: HER4 (ERBB4) Binding Chemiluminescent Assay Kit	82614	96 reactions
Chemi-Verse™ HER2 Kinase Assay Kit	82552	96 reactions

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