Human Amphiregulin ELISA Kit

Cat#: orb735057 (ELISA Manual)

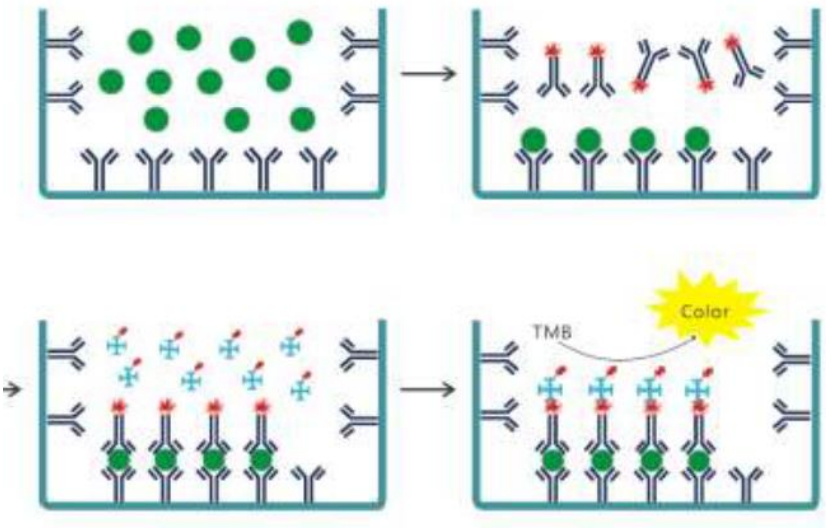
Introduction

Amphiregulin (AR or ARE), also known as schwannoma-derived growth factor (SDGF), is a well-known member of the epidermal growth factor family one. The family also includes TGF-α, HB-EGF, β-animal cellulose (BTC), epiregulin and neuregulin 1-4. All EGF family members are synthesized as type I transmembrane precursors, and they also contain several EGF-like domains in their extracellular regions. Human Amphiregulin is synthesized as a molecule A transmembrane glycoprotein with an amount of about 50kDa, it contains an N-terminal propeptide of 80 amino acids (AA), a heparin binding domain, and an EGF-like knot. Conformation domain, 23 amino acid transmembrane fragment and 31 amino acid cytoplasmic domain. The amino acid sequence of mature human amphiregulin is similar to that of mice and rats. 78% and 76% homology. Some amphiregulin fragments with molecular weights ranging from 9kDa to 43kDa are produced by proteolytic cleavage of transmembrane proteins. pregnancy. The biological activity of amphiregulin is mediated by ADAM17/ TACE and ADAM10. Soluble amphiregulin is released in response to dendrites ATP stimulation of morphocytes and CXCL12 stimulation of prostate epithelial cells. Intracellular distribution of proto-amphiregulin, cell surface shedding and residue fragments the chemistry will be changed by Neisseria's infection of the cells. Amphiregulin produces autocrine and paracrine activities through EGFR and ErbB2. Its interaction with heparan sulfate proteoglycan can increase Strong receptor activation and biological effects. Amphiregulin can act as a mitogen on epithelial cells, keratinocytes, vascular smooth muscle cells and fibroblasts Cell. It plays an important role in the formation of lung and mammary gland branches and PGE-2 induced intestinal epithelial regeneration, and it can also inhibit Fas-mediated liver injury. When an asthma attack occurs, amphiregulin will increase in saliva, and in rheumatoid it will also increase in saliva instead of osteoarthritis synovial fluid. Transmembrane and The soluble form of amphiregulin is upregulated by activated basophils, mast cells and Th2 cells. Amphiregulin promotes the production of inflammatory cytokines and Elimination of nematode infections. In cancer, amphiregulin is upregulated by tumor-associated dendritic cells. It promotes swelling by increasing proliferation, invasion and angiogenesis Tumor progression and also contribute to the resistance of tumor cells to cisplatin. In humans, amphiregulin also promotes oocyte maturation and cumulus cell expansion. HCG exposure will increase in the follicles. It can be upregulated in the reproductive tract of rodents when the embryo is implanted around the uterus and during the second trimester.

Detection principle

This experiment uses a double antibody sandwich ELISA. Pre-coated the ELISA plate with anti-human Amphiregulin monoclonal antibody, add appropriately diluted samples and standards, Among them, Amphiregulin will bind to its monoclonal antibody to wash away free components; adding biotinylated anti-human Amphiregulin antibody, anti-human Amphiregulin antibody binds to human Amphiregulin bound to the monoclonal antibody to form an immune complex, washing away free components; adding horseradish per oxygen Avidin labeled with phytase, biotin and avidin specifically bind to wash away the unbound enzyme conjugate; add color reagent, if there is Amphiregulin horseradish peroxidase will make the colorless

developer turn blue; add stop solution to turn yellow. Measure the OD value at 450nm, Amphiregulin The concentration is proportional to the OD450 value, and the concentration of Amphiregulin in the specimen can be calculated by drawing a standard curve.



Schematic diagram of detection principle

Kit components

Kit components	96 hole	48 hole	Preparation
1a Standard	2 branch	1 branch	Dilute according to the instructions
1b Standards and specimen diluents	1 bottle	1 bottle	Ready to use
2a Concentrated Biotinylated Antibody	2 branch	1 branch	Dilute according to the label
2b Biotinylated Antibody Diluent	1 bottle	1 bottle	Ready to use
3a Concentrated enzyme conjugate (protected from light)	2 branch	1 branch	Dilute according to the label
3b Enzyme Conjugate Diluent	1 bottle	1 bottle	Ready to use
4 Concentrated washing liquid 20×	1 bottle	1 bottle	Dilute according to the label
Chromogenic agent (avoid light)	1 bottle	1 bottle	Ready to use
Stop solution	1 bottle	1 bottle	Ready to use
Antibody coated slats	8×12	8×6	Ready to use
Sealing tape	4 open	2 open	Ready to use
manual	1 share	1 share	

Storage conditions

not yetUnsealed kit		4Store at ℃, please use within the shelf life.
already start seal up or Heavy new Melt untie of try Agent	1b Standards and specimen diluents	Can be put in the whole box 4Store at ℃ 1 Months. 2a Concentrated biotinylated antibodies and 3a Concentrated enzyme conjugate needs to be used and prepared immediately.
	2a Concentrated biotinylated antibodies (100×)	
	2b Biotinylated Antibody Diluent	
	3a Concentrated enzyme conjugate (protect from light 100×)	
	3b Enzyme Conjugate Diluent	
	4 Concentrated washing liquid 20×	
	Chromogenic agent (avoid light)	
	Stop solution	4Store at ℃ or room temperature
	Standard	Repack after re-dissolving,-20Store at ℃ for one month, avoid repeated freezing and thawing. The diluted standard should be discarded after use, and should not be reused.
	Antibody coated slats	The slats not used in the experiment should be put back into the packaging bag immediately, sealed and dried 4Store at ℃.

The above storage conditions are required to be within the shelf life of the kit.

Other experimental materials (Not provided, but can help purchase) **:**
1. Microplate reader (450nm)
2. High-precision adjustable pipette and tip: 0.5-10, 2-20, 20-200, 200-1000μl; It is best to use a multi-channel pipette when testing a large number of samples at a time. Automatic
3. plate washer or bottle washer
4. 37℃ thermostat
5. Double distilled or deionized water
6. Graph paper
7. Measuring cylinder

Precautions

1. The kit is kept in2-8℃, except for the reconstituted standard, other ingredients cannot be frozen. Concentrated Biotinylated Antibody (2a), Concentrated enzyme conjugate (3a)The volume is very small, and the bumps and
2. possible inversion during transportation will cause the liquid to stick to the tube wall or bottle cap. Please centrifuge before use to make the liquid attached to the tube wall or bottle cap settle to the bottom of the tube.
3. To avoid cross-contamination, please use disposable tips.
4. The stop solution and the developer are corrosive. Avoid direct contact with the skin and mucous membranes. Once you come into contact with these liquids, please rinse with plenty of water as soon as
5. possible. Use a clean plastic container to prepare the washing solution, and thoroughly mix the various components and samples in the kit before use.

6. When washing the microtiter plate, pat it dry. Do not put the absorbent paper directly into the microtiter plate to absorb water.
7. Do not use reagents from other sources to mix or replace the components of this product. Kit components of different batch numbers cannot be mixed. Please use this product within the expiry date.
8. In the test, it is recommended to make double or triple wells for standard and sample testing. The order of adding reagents should be the same to ensure that all reaction wells have the same incubation time.
9. Thorough mixing is particularly important for the results of the reaction; it is best to use a micro-oscillator (use the lowest frequency for oscillation).
10. Avoid drying of the microtiter plate during the operation. Drying will quickly inactivate the biological components on the microtiter plate and affect the results of the experiment.
11. Properly dilute the sample so that the sample value falls within the range of the standard curve. According to the different content of the factor to be measured, it is recommended to use1:100, 1:10, 1:2 Dilute the sample. If the sample OD If the value is higher than the highest standard, increase the dilution appropriately and repeat the test.
12. Differences in standard dilutions, operators, pipetting methods, washing methods, incubation time and temperature, and kit batches may cause differences in results.
13. This method can effectively eliminate the interference of soluble receptors, binding proteins and other factors in biological samples.

Sample collection, processing and preservation methods

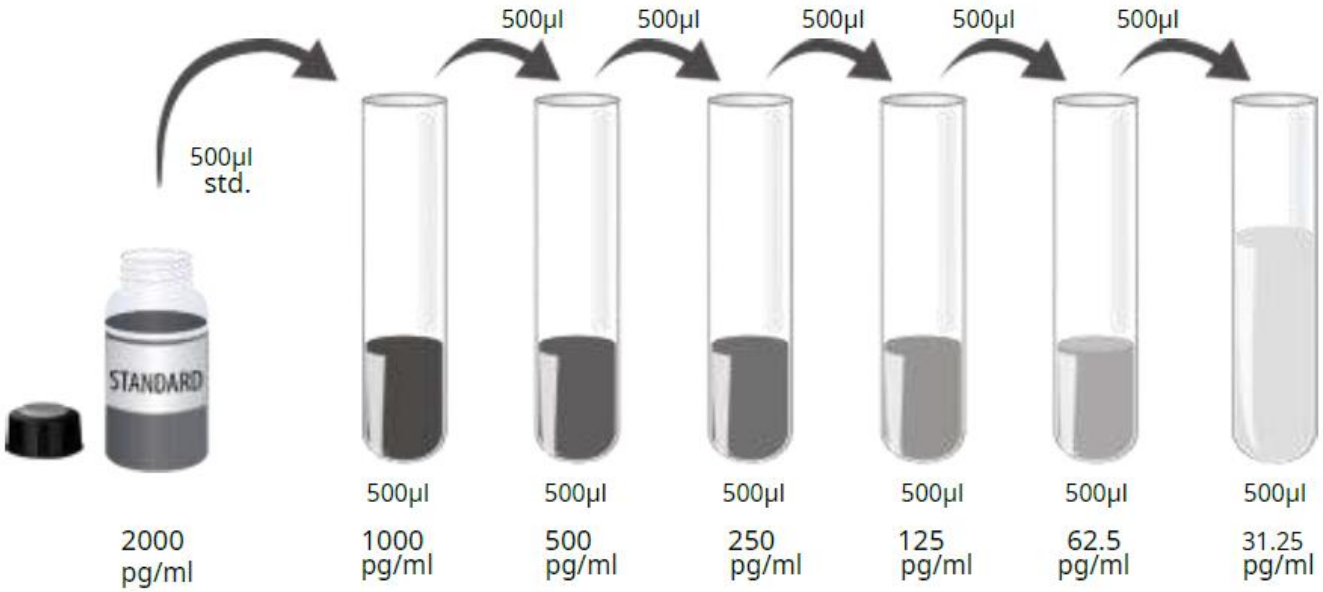
1. **Serum:** Using a pyrogen and endotoxin-free test tube, after collecting the blood, clotting at room temperature30min,1000×gCentrifugal10min, Separate the serum carefully.
2. **plasma**: use EDTA, Citrate and heparin are used as anticoagulants to collect plasma, after collection30minWithin1000×gCentrifugal15minRemove particles.
3. **Cell supernatant**:1000×gCentrifugal10minRemove particles and polymers.
4. **save**: If the sample is not tested immediately, please divide it into one-time use,-20℃-70Store at ℃, avoid repeated freezing and thawing. Try to avoid using hemolytic or hyperlipidemia samples. If the serum contains a lot of particles, centrifuge or filter to remove them before testing; thawing at room temperature, do not37°COr higher temperature heat to thaw.
5. **dilution**: According to the actual situation, the specimen can be diluted by an appropriate multiple (it is recommended to do a preliminary experiment to determine the dilution multiple).
Note: It is recommended to do normal human serum or plasma samples1:2dilution.

Reagent preparation

1. in advance30minRemove the kit from the refrigerator and equilibrate to room temperature.
2. **Washing buffer:** The concentrated washing liquid taken out of the refrigerator may have crystals. This is a normal phenomenon. Heat and gently shake to dissolve the crystals before preparing. Dilute the concentrated washing liquid with double distilled water (1:20). Put back unused 4℃.
3. **Standard:** Add standard/specimen diluent (1b) 1.0mlTo freeze-dried standard (1a)Medium, after dissolving completely, let stand15Minutes to mix (concentration is 2000pg/ml), And then dilute as needed, as shown in

the figure below (it is recommended to use the following concentrations for the standard curve:2000,1000, 500, 250,125, 62.5,31.25, 0 pg/ml). The diluted standard products shall not be reused. The unused standard products should be divided into one-time use and placed in-20~-70Store at ℃, one-time use, avoid repeated freezing and thawing.

Standard dilution method:



4. **Biotinylated antibody working solution:** According to the needs of each hole100µlTo calculate the total amount and prepare more100-200µl. Diluent with biotinylated antibody (2b)Dilute concentrated biotinylated antibody (2a)(1:100). It's best to use it now. (Refer to the table below for the dilution method)

Number of slats used	Concentrated Biotinylated Antibody		Biotinylated Antibody Diluent
12	110µL	+	10890µL
10	90µL	+	8910µL
8	70µL	+	6930µL
6	50µL	+	4950µL
4	33µL	+	3267µL
2	17µL	+	1683µL
1	9µL	+	891µL

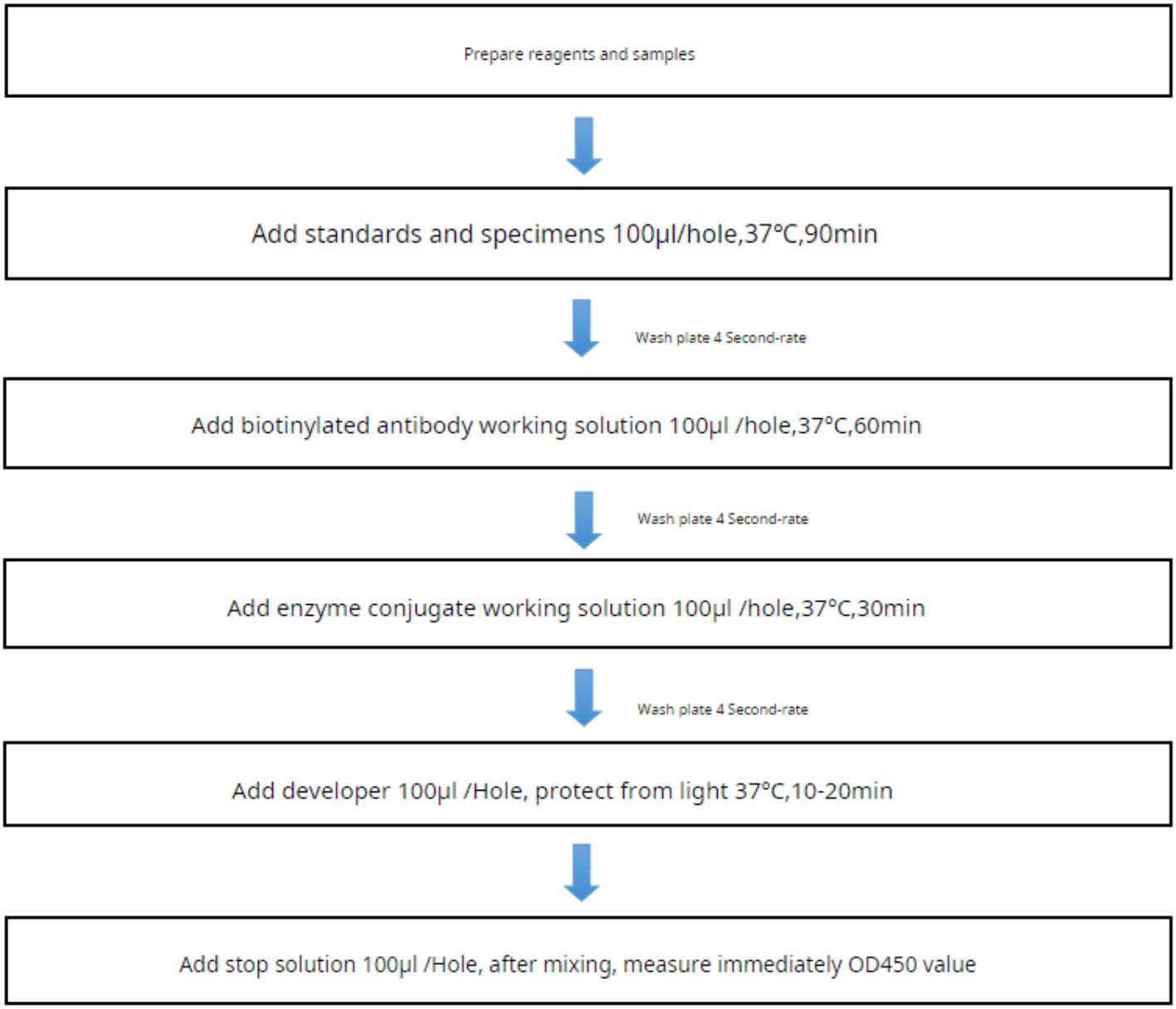
5. **Enzyme conjugate working solution:** Dilute with enzyme conjugate (3b) Dilute concentrated enzyme conjugate (3a)(1:100). It's best to use it now. (Refer to the table below for the dilution method)

Number of slats used	Concentrated enzyme conjugate		Enzyme Conjugate Diluent
12	110μL	+	10890μL
10	90μL	+	8910μL
8	70μL	+	6930μL
6	50μL	+	4950μL
4	33μL	+	3267μL
2	17μL	+	1683μL
1	9μL	+	891μL

Steps

1. Prepare various solutions in accordance with the above preparations.
2. Decide the required number of slats according to the number of samples to be tested and the number of standards, and increase1The hole is used as a blank control hole. Separate the specimens and standards of different concentrations (100μl /Hole) into the corresponding hole (zero hole only add standard/sample diluent), seal the reaction hole with sealing tape,37Incubate at ℃ 90minute **(Except blank control wells)**.
3. Wash plate4Second-rate:(1) Automatic washing machine: the washing liquid required to be injected is350μl, The interval between injection and suction15-30Second. (2) Manual plate washing: Drain all the liquid in the wells, add washing liquid to each well 350μl, Stand still30Shake off the liquid after a second, and pat dry on a thick stack of absorbent paper. Add biotinylated antibody working
4. solution (100μl /hole). Seal the reaction hole with sealing tape,37 Incubate at ℃ 60 minute **(Except blank**
5. **control wells)**. Wash plate4Second-rate.
6. Add enzyme conjugate working solution (100μl /hole). Seal the reaction hole with sealing tape,37Incubate at ℃ 30minute **(Except blank control wells)**.
7. Wash plate4Second-rate.
8. Add developer100μl /Hole, protect from light,37Incubate at ℃10-20minute. Add stop solution
9. 100μl /Hole, measure immediately after mixingOD450 value (5Within minutes).

Operation flow chart



Tips for operation

1. When preparing various reagents, mix them thoroughly, but avoid generating a lot of bubbles, so as to avoid adding a lot of bubbles when adding samples and causing sample addition
2. errors. To avoid cross-contamination, remember to change the tip in time when adding different concentrations of standards, different samples, and different reagents.
3. In order to ensure accurate results, the reaction wells must be sealed with new sealing tape before each incubation. Before adding the color developing agent, it should remain colorless. Do not use the color
4. developing solution that has turned blue. The optimal color development time is very important to the standard curve, which is visible to the naked eye.3-4 The hole has a gradient blue, after 3-4 The hole

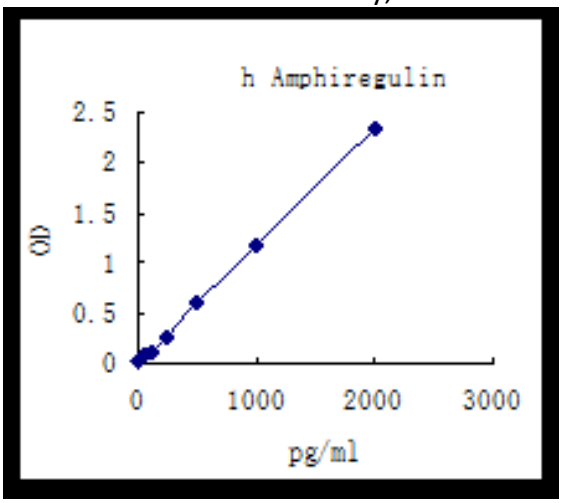
difference is not obvious, and the zero hole can be terminated without blue appearance. A standard curve should be prepared for each test, and the sample should be appropriately diluted or concentrated according to the content
5. of the factor to be tested in the sample. It is best to do a preliminary experiment.

Result judgment

1. Of each standard and specimen OD The value should be subtracted from the blank hole OD Value, if you do duplicate holes, find the average value.
2. Use computer software to measure absorbance OD The value is the ordinate (Y),corresponding Amphiregulin The concentration of the standard is the abscissa (X), Generate the corresponding standard curve, the sample Amphiregulin The content can be based on its OD The value is converted from the standard curve to the corresponding concentration.
3. If the specimen OD If the value is higher than the upper limit of the standard curve, it should be appropriately diluted and re-tested. When calculating the concentration, the sample content should be multiplied by the dilution factor.
4. Reference data:

Standard concentration (pg/ml)	ODvalue1	ODvalue2	average value	Correction value
0	0.036	0.035	0.036	— —
31.25	0.063	0.073	0.068	0.032
62.5	0.082	0.091	0.087	0.051
125	0.134	0.125	0.130	0.094
250	0.262	0.258	0.260	0.224
500	0.598	0.606	0.602	0.566
1000	1.181	1.178	1.180	1.144
2000	2.323	2.327	2.325	2.289

The data is for reference only, the best color rendering time will be different for different users



This figure is for reference only, and should be based on the standard curve drawn by the standard product of the same test

Repeatability of results

The coefficient of variation between the plates and the plates are all <10%.

Sensitivity

Lowest tester Amphiregulin The dose is less than 15pg/ml. Minimum detection method:20 Zero standard average OD The value is increased by two standard deviations, and then the corresponding concentration is calculated.

Specificity

This kit can detect natural and recombinant humans Amphiregulin, by50ng/ml Parallel to do specificity tests, none of them are contrary to the following cytokines and proteins answer.

Recombinant Human Cytokine	Recombinant Mouse Cytokine
EGF	Amphiregulin
EGF R	
HB-EGF	
TGF-α	

References

1. Zeng, F. et al. (2009) Exp. Cell Res. 315:602.
2. Busser, B. et al. (2011) Biochim. Biophys. Acta 1816:119.
3. Xian, CJ (2007) Endocr. Rev. 28:284.
4. Schneider, MR and E. Wolf (2008) J. Cell. Physiol. 218:460.
5. Shoyab, M. et al. (1989) Science 243:1074.
6. Berasain, C. et al. (2005) J. Biol. Chem. 280:19012.
7. Johnson, GR et al. (1993) J. Biol. Chem. 268:18835.
8. Brown, CL et al. (1998) J. Biol. Chem. 273:17258.
9. Lofmark, S. et al. (2011) PLoS ONE 6:e16369.
10. Hinkle, CL et al. (2004) J. Biol. Chem. 279:24179.
11. Sahin, U. et al. (2004) J. Cell Biol. 164:769.
12. Kasina, S. et al. (2009) Cell Prolif. 42:799.
13. Bles, N. et al. (2010) Blood 116:3219.
14. Isokane, M. et al. (2008) J. Cell Sci. 121:3608.
15. Johnson, GR et al. (1993) J. Biol. Chem. 268:2924.