FGF2 ELISA Kit (Cattle) (OKCD02548)

Lot# KD2193

Instructions for use

For the quantitative measurement of FGF2 in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

Variation between lots can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology FGF2 ELISA Kit (Cattle) (OKCD02548) is based on a competitive enzyme immuno assay technique. The microtiter well-plate in this kit has been pre-coated with an anti-Bovine FGF2 antibody. Sample or standards are added to the wells along with a fixed quantity of biotinylated FGF2 and incubated. The FGF2 found in the sample or standards competes with the biotinylated FGF2 for limited binding sites on the immobilized anti-Bovine FGF2 antibody. Excess unbound biotinylated FGF2 and sample or standard FGF2 is washed from the plate. Avidin-HRP conjugate is added, incubated and washed. An enzymatic reaction is then produced through the addition of TMB substrate which is catalyzed by the immobilized HRP to generate a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration is measured by reading the absorbance at 450 nm which is quantitatively proportional to the amount of biotinylated FGF2 captured in the well and inversely proportional to the amount of FGF2 which was contained in the sample or standard.

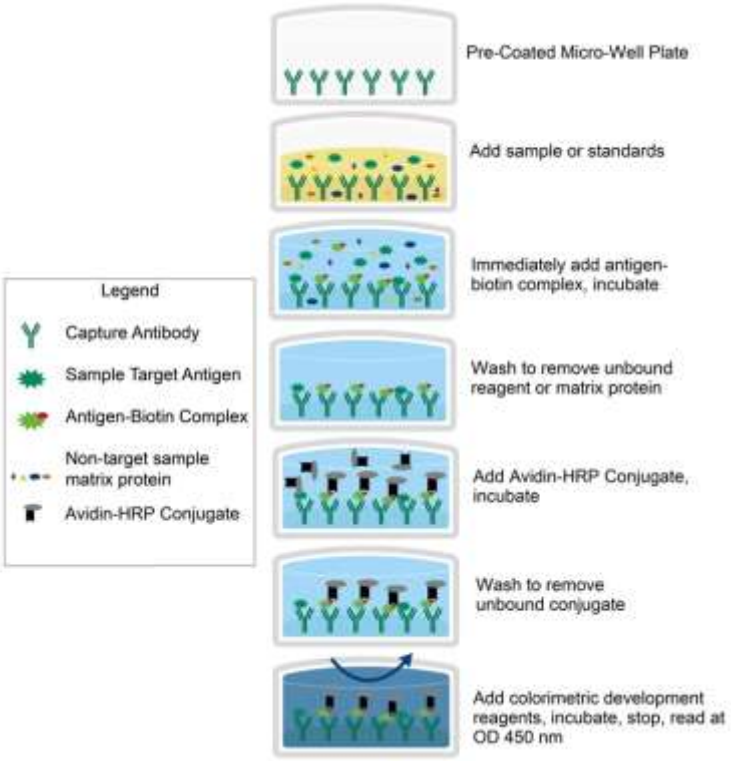
Background

Acts as a ligand for FGFR1, FGFR2, FGFR3 and FGFR4. Also acts as an integrin ligand which is required for FGF2 signaling. Binds to integrin ITGAV:ITGB3. Plays an important role in the regulation of cell survival, cell division, cell differentiation and cell migration. Functions as a potent mitogen in vitro. Can induce angiogenesis.

General Specifications

General Specifications	
Range	12.35 - 1,000 pg/mL
LOD	< 4.54 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Cattle Fibroblast growth factor 2 UniProt ID: P03969 Gene ID: 281161 Target Alias: Fibroblast growth factor 2; Basic fibroblast growth factor; Heparin-binding growth factor 2; BFGF; FGF-2; HBGF-2
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for 6 months or until expiration date. Avoid any freeze/thaw cycles.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Anti-FGF2 Microplate	96 Wells (12 x 8 Well strips)	-20°C for 6 months
FGF2 Lyophilized Standard	2 x 3,000 pg	
FGF2 -Biotin Complex	1 vial (Lyophilized)	
100X Avidin-HRP Conjugate	1 x 120 µL	
Reconstitution Buffer	1 x 300 µL	4°C for 6 months
Biotin Complex Diluent	1 x 12 mL	
Conjugate Diluent	1 x 12 mL	
30X Wash Buffer	1 x 20 mL	
Standard Diluent	1 x 20 mL	
TMB Substrate	1 x 9 mL	
Stop Solution	1 x 6 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

8.1 Cattle FGF2 Assay Standards

8.1.1 Prepare the FGF2 standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one vial of the provided 3,000 pg **Lyophilized FGF2 Standard** for each experiment. Prepare a stock **3,000 pg/mL Standard** by reconstituting one tube of **Lyophilized FGF2 Standard** as follows:

8.1.2.1 Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.

8.1.2.2 Add 1 mL of **Standard Diluent** to the vial.

8.1.2.3 Seal the vial then mix gently and thoroughly.

8.1.2.4 Leave the vial at ambient temperature for 15 minutes.

8.1.3 Prepare a set of serially diluted standards as follows:

8.1.3.1 Label tubes with numbers 1 – 8.

8.1.3.2 Add 600 µL of **Standard Diluent** to Tube #'s 2 – 8.

8.1.3.3 Prepare a **1,000 pg/mL Standard #1** by adding 300 µL of **3,000 pg/mL Standard** to 600 µL of **Standard Diluent** in Tube #1. Mix gently and thoroughly.

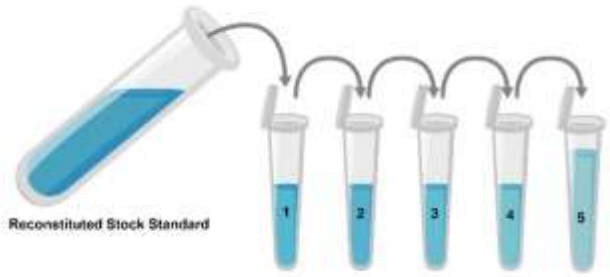
8.1.3.4 Prepare **Standard #2** by adding 300 µL of **Standard #1** (Tube #1) to Tube #2. Mix gently and thoroughly.

8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #8 is a blank standard (only **Standard Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Standard Diluent (µL)	Total Volume (µL)	Final Concentration
1	3,000 pg/mL Reconstituted Standard	300	600	900	1,000 pg/mL
2	1,000 pg/mL	300	600	900	333.33 pg/mL
3	333.33 pg/mL	300	600	900	111.11 pg/mL
4	111.11 pg/mL	300	600	900	37.04 pg/mL
5	37.04 pg/mL	300	600	900	12.35 pg/mL
6	NA	0	600	900	0.0 (Blank)



8.2 1X FGF2 Biotin Complex

8.2.1 Immediately prior to use, first prepare a **100X FGF2-Biotin Complex** by adding 150 µL of **Reconstitution Buffer** to the vial of **FGF2-Biotin Complex**. Mix gently and thoroughly. Use immediately.

8.2.2 Dilute the **100X FGF2-Biotin Complex** 1:100 with **Complex Diluent** to prepare a **1X FGF2-Biotin Complex** as follows:

8.2.2.1 For each well strip to be used in the experiment (8-wells) prepare 500 µL by adding 5 µL of **100X FGF2-Biotin Complex** to 495 µL **Complex Diluent**.

8.2.2.2 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.3 1X HRP-Avidin Conjugate

8.3.1 Prepare the **1X Avidin-HRP Conjugate** immediately prior to use by diluting the **100X Avidin-HRP Conjugate** 1:100 with **Conjugate Diluent**.

8.3.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Avidin-HRP Conjugate** to 990 µL **Conjugate Diluent**.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4 1X Wash Buffer

8.4.1 If crystals have formed in the **30X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.4.2 Add the entire 20 mL contents of the **30X Wash Buffer** bottle to 580 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.4.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

• Samples not indicated in the manual must be tested to determine if the kit is valid.

• Prepare samples as follows:

• **Serum** - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at 1,000 x g. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• **Tissue Homogenates** – Rinse 100 mg tissue with 1X PBS then homogenize in 1 mL of 1X PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes then centrifuge for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

• **Cell Lysates** – Cells need to be lysed before assaying according to the following directions:
1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.
3. Resuspend cells in fresh lysis buffer with concentration of 10^7 cells/ml. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤ -20°C.

• **Cell culture supernatants and other biological fluids** - Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

9.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting no signal should be further diluted (note the inversely proportional signal of the competitive assay).

• Dilute samples using **Standard Diluent**.

• Mix diluted samples gently and thoroughly.

• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Retain at least one well as an absolute Blank without any samples or reagents.

10.3 Add 50 µL of serially titrated standards, diluted samples or blank into wells of the **Anti-FGF2 Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.4 Immediately add 50 µL of **1X FGF2-Biotin Complex** to each well (excluding absolute Blank).

10.5 Cover the plate with the plate sealer and incubate for 60 minutes at 37°C.

10.6 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.7 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time

10.8 Wash plate four times with **1X Wash Buffer** as follows:

10.8.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.8.2 Incubate for 1-2 minutes.

10.8.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.8.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.8.5 Repeat steps 10.8.1 through 10.8.4 **three** more times.

10.9 Add 100 µL of **1X Avidin-HRP Conjugate** to each well.

10.10 Cover the plate with the plate sealer and incubate at 37°C for 30 minutes.

10.11 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.12 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.13 Repeat wash as in **step 10.8.**

10.14 Add 90 µL of **TMB Substrate** to each well, cover with plate sealer and incubate at 37°C **in the dark** for 10-20 minutes. Wells should change to gradations of blue. If the color is too deep based on the standard, adjust incubation times.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the bottom four standard wells, while the remaining standards still appear clearer.)

10.15 Add 50µL of **Stop Solution** to each well. Well color should change to gradations of yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.

10.16 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.15. If wavelength correction is available, set to 540 nm or 570 nm.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

(Relative OD_{450}) = (Well OD_{450}) – (Mean Blank Well OD_{450})

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The **FGF2** concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

Note: If wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

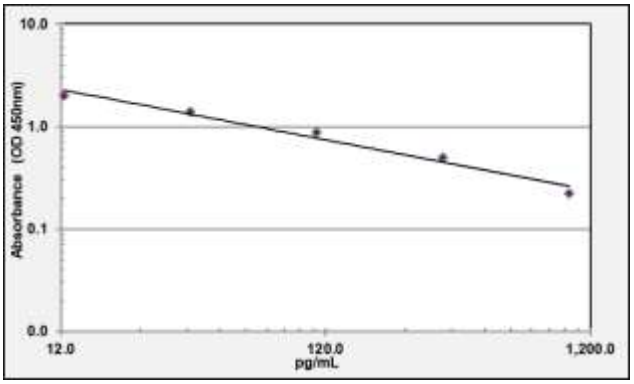
12.1 Reproducibility

Intra-assay Precision: 3 samples with known low, middle and high levels FGF2 were tested with 20 replicates on one plate, respectively. Inter-assay Precision: 3 samples with known low, middle and high level FGF2 were tested on 3 different plates, 8 replicates in each plate.

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/ml)	37.42	173.21	430.24	38.55	174.56	441.23
SD	2.395	9.007	20.652	2.737	10.124	20.738
CV (%)	6.4	5.2	4.8	7.1	5.8	4.7

12.2 Typical standard curve

This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



pg/mL	Absorbance		Mean Absorbance	Log of Concentration
	Rep 1	Rep 2		
1000	0.223	0.225	0.224	3.000
333.33	0.499	0.497	0.498	2.523
111.11	0.886	0.872	0.879	2.046
37.04	1.391	1.385	1.388	1.569
12.35	1.988	2.014	2.001	1.092

12.3 Linearity

Kit linearity evaluated by testing serially diluted samples containing known concentrations of FGF2. Results are expressed as the percentage of the expected concentration measurement.

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	84-93%	90-104%	85-92%	89-101%
EDTA Plasma (n=5)	79-97%	94-107%	80-95%	86-99%
heparin Plasma (n=5)	80-90%	81-96%	83-98%	78-94%

12.4 Recovery

The following matrices were spiked to known concentrations using recombinant FGF2. Recovery is expressed as the percentage of the expected concentration measurement.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	82-95	88
EDTA plasma (n=5)	97-105	101
Heparin Plasma (n=5)	83-97	91

13. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

Aviva Systems Biology, Corp.
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Phone: 858-552-6979
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