Ab-Match ASSEMBLY Human FAM3D kit

Catalog Number: 5321

*Note
Ab-Match ASSEMBLY Human FAM3D kit should be stored at -20°C.

For technical material or related information, please refer to https://ruo.mbl.co.jp/product/cancer/abmatch.html.

■ Advantage

Ab-Match ASSEMBLY Human FAM3D kit contains analyte specific reagents for the measurement of human FAM3D. The kit comprises of the capture antibody for coating, the biotin conjugated detection antibody, peroxidase conjugated streptavidin and the concentration standard. Additional reagents are required to build and ELISA with the Ab-Match ASSEMBLY Human FAM3D kit. Those reagents comprise of the Ab-Match UNIVERSAL kit. Please use these two kits in combination in order to build and ELISA to quantify human FAM3D in suitable biological samples.

The two kits are sufficient to produce one 96 well microplate. Overnight incubation (14 hours plus 1 hour for blocking to coat the plate) is required to prepare the ELISA plate. Another 3 hours are needed to perform the assay and measure human FAM3D.

This product is intended for research use only.

■ FAM3D

FAM3D (Family with sequence similarity 3, member D, also called EF-7) has been suggested to be new kind of cytokine, because it has a 4-helix bundle structure, which is thought to be unique to growth factors and cytokines. The FAM3 family, consists of four proteins. The receptors and functions of FAM3A, FAM3B, FAM3C are unknown. While FAM3B is found in the pancreas, FAM3D is expressed in the placenta, and FAM3A and FAM3C are ubiquitously expressed (Ref1).

The expression of FAM3D is reported to increase in colon cancer patients (Ref2). MBL's FAM3D ELISA kit can detect FAM3D protein in the culture supernatants of the colon cancer derived cell lines LOVO and WiDr.

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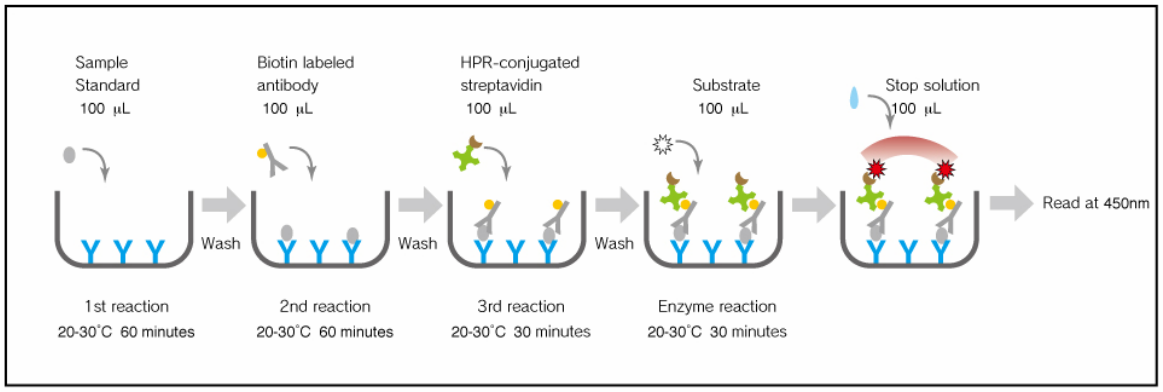
■ Assay principle



The anti-FAM3D capture antibody is coated on a 96 microwell plate. Standards or samples are added to the microwells, allowing FAM3D to bind to the capture antibody in proportion to the concentration of FAM3D in the sample (primary reaction). After wash unbound FAM3D, biotin conjugated anti-FAM3D detection antibody is added and attaches to the FAM3D bound to coated antibody (secondary reaction).

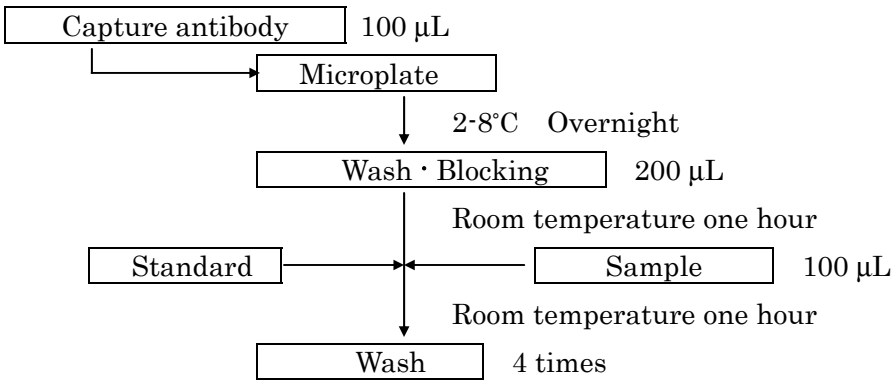
After washing away all unbound detection antibody, peroxidase conjugated streptavidin (SA-HRP) is added and attaches to the biotin label on the bound detection antibody (biotin-avidin reaction). Another wash removes all unbound SA-HRP, before the substrate is added the peroxidase reaction.

After adding the stop solution to the microwell plate, the relative amount of FAM3D bound in each well can be determined from the yellow color using a microplate reader. The concentration of FAM3D in each sample can be obtained by comparing the OD of the sample to the OD of the standards on the plate.

■ Procedure

Prepare and use the Ab-Match ASSEMBLY kit reagent's standard and antibody solution with Ab-Match UNIVERSAL kit's buffer solution. Ab-Match UNIVERSAL kit contains the buffer solutions, microwell strips, assay diluent, substrate solution, and stop solution required to prepare one 96 well ELISA using one Ab-Match ASSEMBLY kit.

1) Procedure : outline



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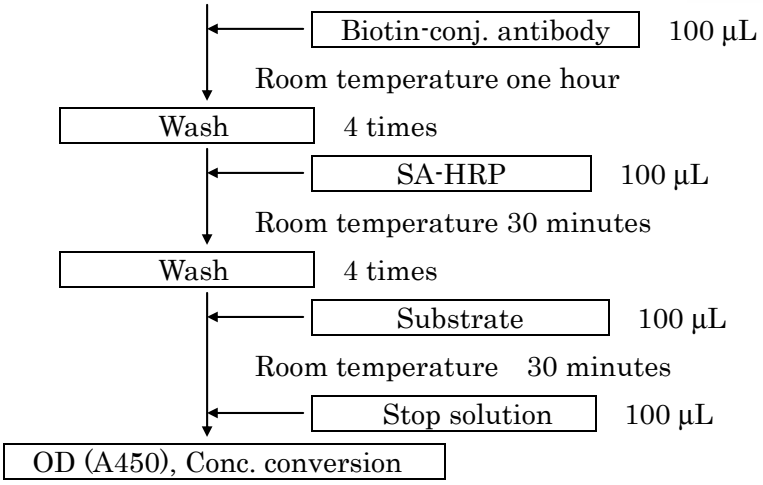

Standard, each antibody, and SA-HRP should be prepared according to preparation method shown below.

2) REAGENTS AND PREPARATION

1 x 96 well

For reagents highlighted in a box (,) use Ab-Match UNIVERSAL kit components. Each vial should be centrifuged prior to use in order to collect the reagent that is on the wall and cap of each vial.

Component	Volume	No.	Form	Storage
FAM3D standard	200 µL*	1	solution	-20°C
	*The concentration is printed to the label.			
Human FAM3D recombinant ・ Must be stored frozen. After thawing frozen FAM3D standard aliquote and store at -20°C (stock solution). ・ For the assay procedure, stock solution should be diluted 1: 10 with Sample Diluent or culture medium ・ With the 1:10 dilution as top standard, 5 to 7 standards should be made with Sample Diluent or culture medium by 2-fold serial dilution. Use the same diluent without any standard added as blank (zero conc.).				
Anti -FAM3D capture antibody	60 µL	1	solution	-20 °C
Mouse monoclonal antibody (IgG) 1mg/mL (50% Glycerol/PBS) ・ Prepare 1:200 dilution using Coating Buffer for antibody coating ・ Dilution must be used immediately and cannot be stored for future use.				
Anti-FAM3D detection antibody (biotin conj.)	110 µL	1	solution	-20 °C
Biotin labeled mouse monoclonal antibody ・ Prepare 1: 100 dilution using Sample Diluent . ・ Dilution must be used immediately and cannot be stored for future use.				
Streptavidin conj. peroxidase (SA-HRP)	110 µL	1	solution	-20 °C
・ Prepare 1:100 dilution using SA-HRP Diluent . ・ Dilution must be used immediately and cannot be stored for future use.				

Note) Kit components must be brought to room temperature (20-30 °C) prior to use.

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Ab-Match UNIVERSAL kit component (available separately, Catalog Number: 5310)

Microwell strips	96 wells	1		RT/2-8 °C
Not coated				
Coating Buffer	12 mL	1	solution	2-8 °C
Carbonate buffer solution				
Blocking Agent	24 mL	1	solution	2-8 °C
Contains BSA and sucrose				
Sample Diluent	50 mL	1	solution	2-8 °C
Contains BSA, Tween 20 and HAMA-Blocker				
Wash Concentrate (PBS/Tween 20) (20X)	50 mL	1	solution	2-8 °C
Buffer solution containing Tween 20 Wash Solution : Prepare 1 : 20 dilution of The Wash Concentrate prior to use (ex. Add 50 mL of Wash Concentrate to 950 mL of deionized water). After preparation, stable for 2 weeks at 2-8 °C.				
SA-HRP Diluent	15 mL	1	solution	2-8 °C
Contains BSA				
Substrate Solution	20 mL	1	solution	2-8 °C
Tetramethylbenzidine (TMB) / H_2O_2 solution				
Stop Solution	20 mL	1	solution	2-8 °C
0.25 mol/L sulfuric acid				

* Except Wash Concentrate, all are ready to use.
* RT indicates room temperature.

3) Microplate Coating with Capture Antibody

For reagents stated in bracket, use components of the Ab-Match UNIVERSAL kit (available separately).

1. Dilute Antibody for coating 1:200 with Coating Buffer. Soon after mixing by repeated inversion, dispense 100 µL to each microwell with multichannel pipette and let stand overnight at 2-8 °C with seal(or other cover to avoid evaporation).
   * Use a new conical 15ml tube to mix the antibody solution.
2. Aspirate and discarding the antibody solution, wash with saline two times.
   * saline : NaCl 9.0g / 1,000 mL
   For washing method, refer to Assay procedure 4 .
3. Add 200 µL of Blocking Agent to each well and incubate for one hour at room temperature. Dump out the contents of the wells over sink before use.
   * Aspiration of antibody and addition of blocking should be done as soon as possible as to not to let the cup become dry.
   * After completion of the blocking step, the coated plate can be stored for a long period of time when properly dried. After aspirating and discarding blocking

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agent, apply fan or other drying method and leave at room temperature for 3 hours to overnight to ensure proper drying. Store the plate at 2-8 ℃, under strictly controlled moisture conditions. The color development of dried plates may be decreased when compared with plates that were never dried.

4) Assay procedure

1. Dilute standard stock solution 1:10 with Sample Diluent or culture medium. With this as top standard, 5 to 7 standards should be made with Sample Diluent or culture medium through 2-fold serial dilution. Then use buffer solution used for preparation as zero conc.(Blank).
   We recommend to use standards in duplicate wells, which requires 200 ul of each standard solution,
2. Dilute sample with optimal dilution in Sample Diluent or culture medium.
   * For human serum, 1: 40 dilution is recommended.
   * When assayed with culture supernatant as sample, culture medium should be used to dilute the standard.
   * Sample Diluent contains HAMA-Blocker to block the effect of human anti- mouse antibodies (HAMA) which may be present in human serum.
3. Add 100 µL of each diluted standard solution and sample to an antibody coated well on the plate. Incubate for one hour at room temperature (primary reaction)
   * The Antigen-antibody reaction starts on addition. Addition should be completed as quickly as possible. It is recommended that standard and sample are diluted on a separate microplate in advance, then added to the antibody coated plate with a multichannel pipette.
   * When adding solution to the microplate, avoid touching the inner wall of microcup with the pipet tip. This technique avoids non-specific reaction.
4. Wash four times with Washing Solution.
   Washing method)
   Put washing solution in wash bottle. Hold plate over the sink upside-down and shake once to discard the liquid out of the wells. Be careful not to shake too strong because strip fixing may be detached from microcup frame, but strong enough to discard most of the liquid out from each well.
   Gently pour wasing solution from the wash bottle into the empty wells. Repeat the discarding and refilling steps.
   Finally, tap the plate several times on several piles of clean paper towel to remove any washing solution that remained in the wells. Any remaining washing solution would lead to dispersion.
   * If an autowasher is used, optimal washing times vary depending on the instrument used and its setting.
   * Wash as quickly as possible, DO NOT let wells dry up!
5. Add 100 µL of diluted biotin labeled detection antibody to each well. Incubate for one hour at room temperature.
   * Avoid touching the inner wall of the microcups with the pipette tip. Otherwise, it could cause of non-specific reaction.
6. Wash following step 4 above.
7. Add 100 µL of diluted SA-HRP solution to each well. Incubate for 30 minutes at room temperature.

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＊ Avoid touching the inner wall of the microcups with the pipette tip. Otherwise, it could cause of non-specific reaction.

8. Wash following step 4 above.
9. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature.
   ＊ The mircoplate will change to blue.
10. Add 100 µL of Stop Solution to each well.
   ＊ The microplate wlll change to yellow.
   ＊ Read absorbance within 30 minutes after reaction stop.
11. Using microplate reader, read the absorbance of each well at test wavelength of 450 nm. In addition, the wavelength of 620 nm may be measured.

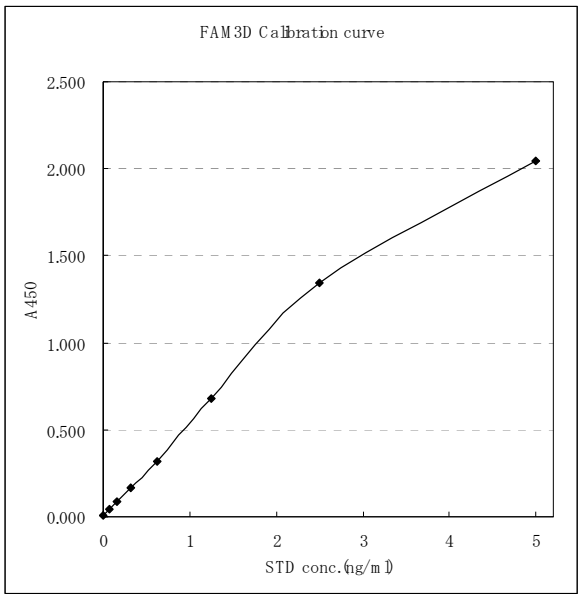
5) Calculation of result

Calculate the mean absorbance value of each standard and plot against log standard concentration and connect the points the best fitting straight line. The concentration of the samples then can be read from this standard curve. Alternatively a suitable computer and curve-fitting program can be used. The concentration read from the standard curve must be multiplied by the dilution factor.

＊ If a sample's O.D. is out of range for the standard curve, the assay should be repeated with a higher sample dilution. ODs should always remain below 2.0 in order to remain in the dynamic range of the detection system.
＊ When computer software is used, logistic, 3 (4) -para-logit-log, or Spline may be used as calculation.
＊ A concentration conversion spreadsheet using 4-para-Logistic regression with Excel 2000 (Microsoft) is available from https://ruo.mbl.co.jp/product/cancer/abmatch.html.

■ Calibration Curve

This Calibration Curve is used only for demonstration purposes.



■ Reference data

1. Intra-assay

ng/mL	CV%
0.183	3.0
0.350	1.2
0.627	2.1
1.215	3.7
2.511	1.7
4.993	2.9

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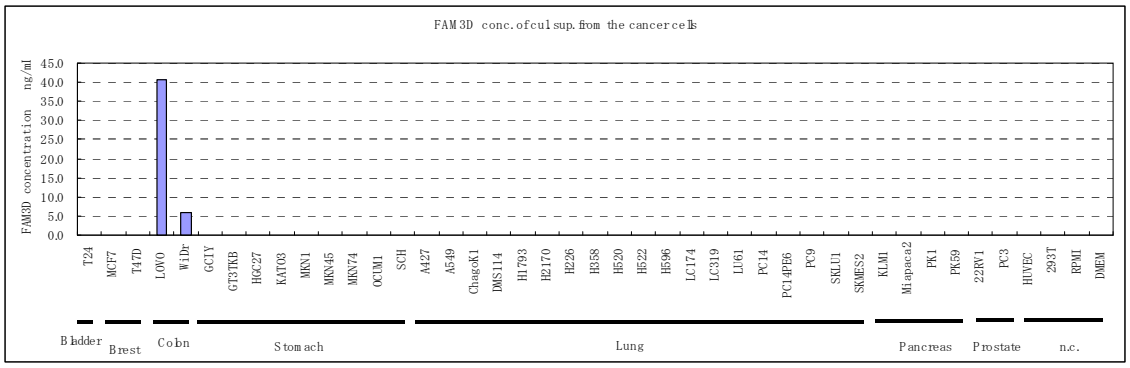
2. Detection Limit

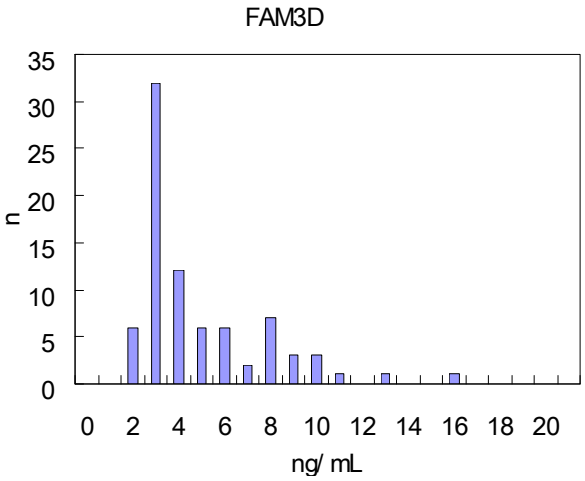
The detection limit was calculated on zero calibrator replicates (n=8) and was 7 pg/mL. By interpolating the imprecision profile with coefficient of variation of 10% was 40 pg/mL.

3. Reactivity with established cancer cell line culture supernatant



4. Normal range (95% reference interval)

The reference interval was 1.6 to 12.0 ng/mL in the serum from healthy samples (n=80).



■ PRECAUTIONS

- Do not use reagents beyond the stated expiration dates.
- One Standard curve per assay should be made.
- Operation for dispensing and diluting should be precisely done.
- Avoid contact of substrate solution, stop solution with skin or eyes. If contacted, wash away with plenty of water.
- Substrate solution is easily oxidized with metal ions. Use disposable new instruments and disposable pipettes for all handling of the substrate solution Never return substrate solution to the substrate reagent bottle!

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- Serum samples may be infectious. Instruments used in this test should be disposed after use or treated as follows:
  Soak in 2% final conc. glutaraldehyde solution for more than one hour or soak in 0.5% Sodium hyperchloride solution (available chloric: approx. 5,000 ppm) for more than one hour or autoclave at 121˚C for more than 20 min.
- The incubation times indicated do not allow the incubation to complete. Frequent moving of the plate during standing, or vibration from instruments, may cause a shaking effect to to the reaction solution, causing the reaction to progresses faster than usual and giving higher color development.

■ STORAGE

Stored at -20 ~ -30 ˚C

■ SHELF LIFE

12 months after shipment

■ RELATED PRODUCTS

Ab-Match UNIVERSAL kit　Catalog Number: 5310

■ REFERENCES

Ref1: Genomics 2002 80 p144-p150
Ref2: Int J Oncol. 2004 25(4) p1049-p1056

■ MANUFACTURER

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