

XIII Fibrinogen

Fibrinogen is the main protein of blood coagulation system. It is a large protein (MW 340 kDa) and it consists of two identical subunits that contain three polypeptide chains: α , β and γ . All chains are connected with each other by a number of disulfide bonds. Fibrinopeptides A (1 – 16 amino acids) and B (1 – 17 amino acids) are released by thrombin from the N-terminal parts of α - and β -chains, respectively. In this way fibrinogen is converted into fibrin, which by means of polymerization forms a fibrin clot. Fibrinogen clotting underlies pathogenesis of MI, thromboembolism and thromboses of arteries and veins, since fibrin is the

main substrate for thrombus formation. Fibrinogen activation is also involved in pathogenesis of inflammation, tumor growth and many other diseases.

The normal fibrinogen concentration in plasma is about 3 mg/ml. The elevated level of fibrinogen in patient's blood is regarded as an independent risk factor for cardiovascular diseases. An increase in blood fibrinogen concentration was shown to be a strong predictor of coronary heart disease (1, 2). All these facts make fibrinogen an important parameter in the diagnosis of cardiovascular diseases.

1. Anti-fibrinogen monoclonal antibodies

Host animal: mice BALB/c
Cell line used for fusion: Sp2/0
Antigen: Fibrinopeptide A or human fibrin degradation products
Purification method: protein A affinity chromatography
Presentation: MAb solution in PBS with 0.1 % sodium azide
Application: Fibrinogen immunoassay and Fibrinogen immunodetection in Western Blotting.

Hybridoma cell lines producing MABs were derived from hybridization of SP2/0 myeloma cells with spleen cells of BALB/c mice immunized with purified human fibrin degradation products or fibrinopeptide A.

Specificity of antibodies was confirmed by ELISA and Western Blotting. All antibodies recognize fibrinogen in ELISA. MABs 1F3, 6G12, 15E11, 27C8 and 40F11 recognize fibrinogen in Western Blotting after SDS gel electrophoresis under non-reducing conditions; MABs 15H12 and 41D9 recognize fibrinogen in Western blotting after electrophoresis under both reducing and non-reducing conditions (Fig. 35).

Antibodies recommended for sandwich immunoassay to detect fibrinogen together with fibrin degradation products are:

1F3 ——— 27C8
 40F11 ——— 1F3

For specific fibrinogen detection see section XIV (fibrinopeptide A).

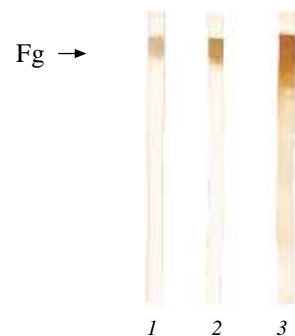


Fig. 35. Detection of fibrinogen by anti-fibrinogen antibodies in Western Blotting (non-reducing conditions). Lane 1 - MAb 1F3; Lane 2 - MAb 6G12; Lane 3 - MAb 15H12

Ordering information

Clone	Cat.#	Specificity	Subclass	Application
1F3	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
6G12	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
15E11	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
15H12	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
27C8	4F1	Fibrinogen, Fibrin degradation products	IgG2a	EIA, Sandwich immunoassay (capture, detection), WB
40F11	4F1	Fibrinogen, Fibrin degradation products	IgG1	EIA, Sandwich immunoassay (capture, detection), WB
41D9	4F1	Fibrinogen, Fibrin degradation products	IgG2a	EIA, Sandwich immunoassay (capture, detection), WB

References:

1. Lowe G. et al. *Blood rheology, cardiovascular risk factors, and cardiovascular disease: the West of Scotland Coronary Prevention Study. Thromb Haemost*, 2000; 84:553-8.
2. Danesh J. et al. *Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA*, 1998; 279:1477-82 <http://www.researchd.com/absort.htm>.

XIV Fibrinopeptide A

Fibrinogen consists of two identical subunits that contain three polypeptide chains: α , β and γ . The process of fibrinogen clotting begins with removal of N-terminal peptides from its α -chains (fibrinopeptide A) and β -chains (fibrinopeptide B) by thrombin that is formed by a set of cascade reactions. The increase of fibrinopeptide A level is a direct marker of fibrinogen clotting. Elevated levels of fibrinopeptide A were found in patients with myocardial infarction, coronary heart disease and other disorders accompanied by the activation of blood coagulation system.

Fibrinogen can be specifically detected by immunological methods only if capture antibodies recognize fibrinopeptides A or B in intact fibrinogen. Otherwise fibrin degradation products will be measured together with fibrinogen. It is not important when fibrinogen is measured in healthy individuals or in patients with mild hypercoagulation but becomes of great significance in disorders with disseminated intravascular coagulation (DIC) when the amount of fibrin degradation products in blood is comparable with the amount of fibrinogen.

1. Synthetic fibrinopeptide A

Source: amino acid synthesis
Purity: > 99 % by SDS-PAGE
Presentation: lyophilized
Application: immunoassay standard and calibrator

Fibrinopeptide A is synthesized from amino acids according to the sequence ADSGEGDFLAE GGGVR that is 1–16 amino acids of the α -chain of fibrinogen.

The identity and purity of fibrinopeptide A was confirmed by mass spectroscopy. The purity was found to be more than 99 %.

Ordering information

Product	Cat. #	Purity	Source
Synthetic fibrinopeptide A	8FP1	>99 %	Amino acid synthesis

2. Anti-fibrinopeptide A monoclonal antibodies

Host animal: mice BALB/c
Cell line used for fusion: Sp2/0
Antigen: Fibrinopeptide A
Purification method: protein A affinity chromatography
Presentation: MAb solution in PBS with 0.1 % sodium azide
Application: Fibrinogen and fibrinopeptide A immunoassay

Hybridoma cell lines producing MAbs were derived from hybridization of SP2/0 myeloma cells with spleen cells of BALB/c mice immunized with fibrinopeptide A conjugated with a carrier protein. Antibodies recognize free fibrinopeptide A and fibrinopeptide A region of fibrinogen α -chain.

immunoassay we recommend using anti-fibrinopeptide A MAbs as capture and anti-fibrinogen MAbs as detection. Recommended pairs:

49D2 (anti-fibrinopeptide A) — 40F11 (anti-fibrinogen)
1F7 (anti-fibrinopeptide A) — 1F3 (anti-fibrinogen)
26E7 (anti-fibrinopeptide A) — 27C8 (anti-fibrinogen)

For specific fibrinogen detection by sandwich

Ordering information

Clone	Cat. #	Specificity	Subclass	Application
1F7	4FP1	Fibrinopeptide A	IgG2a	EIA, Sandwich immunoassay (capture)
26E7	4FP1	Fibrinopeptide A	IgG2b	EIA, Sandwich immunoassay (capture)
49D2	4FP1	Fibrinopeptide A	IgG2a	EIA, Sandwich immunoassay (capture)

1. Sonel A. et al. *Prospective Study Correlating Fibrinopeptide A, Troponin I, Myoglobin, and Myosin Light Chain Levels With Early and Late Ischemic Events in Consecutive Patients Presenting to the Emergency Department With Chest Pain.* *Circulation.* 2000; 102:1107-13.
2. Rapold H.J. et al. *Fibrin formation and platelet activation in patients with myocardial infarction and normal coronary arteries.* *Eur Heart J.* 1989; 10:323-33.

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TROPONIN I

TROPONIN T

TROPONIN C

MYOGLOBIN

FATTY ACID BINDING GLOBULIN

GLYCOGEN PHOSPHORYLASE, BB ENZYME (GPBB)

C-Reactive Protein

BRAIN S-100 Protein

ANTIBODIES, Antigens and more.....

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