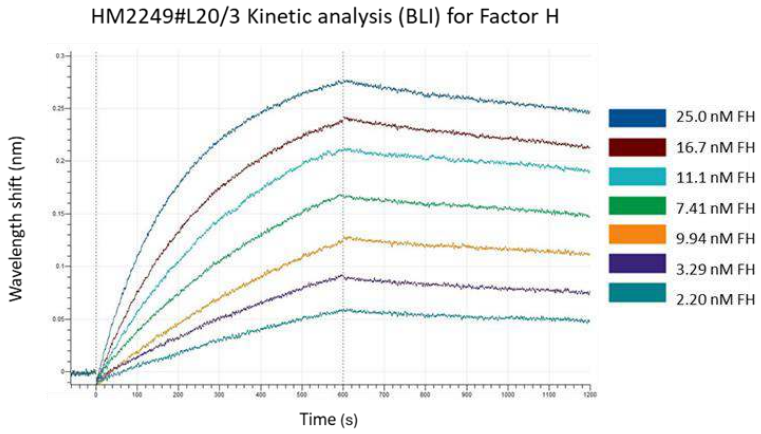
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	FH & FHRs, Human, clone L20/3		
Catalog number	HM2249-100UG		
Lot number	-	**Expiry date**	-
Volume	1 ml	**Amount**	100 µg
Formulation	0.2 µm filtered in PBS+0.1%BSA+0.02%NaN3	**Concentration**	100 µg/ml
Host Species	Mouse IgG1	**Conjugate**	None
Endotoxin	N.A.	**Purification**	Protein G
Storage	4°C		

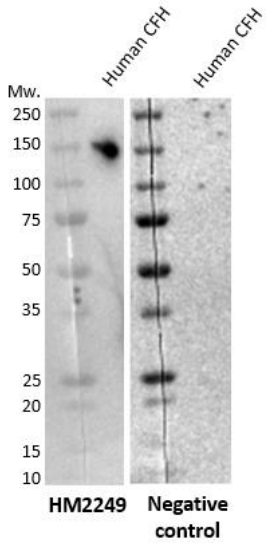
Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	BLI	W
Reference #									
Yes						•		•	•
No									
N.D.	•	•	•	•	•		•		

N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; BLI = Bio-Layer Interferometry, W = Western blot



Protein	ka (1/Ms)	kdis (1/s)	KD (M)	R^2
Factor H	8.6E04	1.3E-04	1.1E-09	0.9972
FHR-1	6.0E05	1.7E-06	2.9E-12	0.9990
FHR-2	2.2E05	6.6E-03	3.0E-08	0.9888
FHR-3	-	-	NA	-
FHR-4	4.6E05	5.6E-05	1.2E-09	0.9206
FHR-5	1.6E06	3.6E-04	2.2E-10	0.9986



BLI: The kinetic parameters of antibody HM2249 were determined for native human Factor H and recombinant proteins FHR-1, FHR-2, FHR-3, FHR-4 and FHR-5 using BLI (Octet R8). The HM2249 antibody was immobilized on streptavidin sensors and kinetic parameters were measured against the proteins in solution. Curve fitting and calculations of kinetic parameters were executed in a 1:1 model using the Octet Analysis Studio Software version 13.0.2.46. The graph represents the association-dissociation curves for HM2249 against Factor H.

W: Western blot analysis was performed for native human Factor H under non-reduced conditions. HM2249 antibody was applied at 2 µg/ml. Expected band size CFH: 155 kDa.

Dilutions to be used depend on detection system applied. It is recommended that users test the reagent and determine their own optimal dilutions. The typical starting working dilution is 1:50.

General Information

Description

Monoclonal antibody HM2249 clone L20/3 recognizes human complement factor H and FHR proteins (FHR-1, FHR-2, FHR-4 and FHR-5) through recognition of the SCR20 domain.

The complement system is a key component of the immune response that defends against infections, initiates inflammation, manages immune complexes and regulation of immune responses. Complement factor H (CFH or FH) is the principal regulator of the alternative pathway, ensuring that complement activation is restricted to appropriate targets. Unlike the classical and lectin pathways, the alternative pathway is constitutively active at a low level and therefore requires tight control. CFH exerts this control by binding to C3b, accelerating decay of the C3 convertase (C3bBb), and acting as a cofactor for Complement factor I-mediated cleavage of C3b. In addition, CFH inhibits convertase formation by competing with factor B. Through these mechanisms, it regulates complement both in fluid phase and on host cell surfaces.

Structurally, CFH is a ~150 kDa plasma glycoprotein composed of linearly arranged 20 short consensus repeat (SCR) domains (also called complement control protein (CCP) modules). These domains confer multiple ligand-binding sites, including three regions for C3b and several for polyanions such as glycosaminoglycans. Functionally, CFH is organized into two main regions: the N-terminal SCR1–4 domains mediate complement regulatory activity, while the C-terminal domains, particularly SCR19–20 with contribution from SCR7, enable recognition of host surfaces via binding to C3 fragments and polyanions. This dual architecture allows CFH to selectively protect self-tissues from complement-mediated damage.

CFH is part of a protein family that includes the complement factor H-related (FHR) proteins (FHR-1, FHR-2, FHR-3, FHR-4, FHR-5), which share a similar SCR-based structure and high sequence homology, particularly in domains corresponding to CFH surface-recognition regions. Unlike Factor H-like protein 1 (FHL-1), FHR-1 - 5 proteins lack domains equivalent to CFH SCR1–4 and therefore generally lack intrinsic complement regulatory activity. Instead, they are thought to modulate complement by competing with CFH for ligand binding.

Complement factor H-related protein 1 shows the closest similarity to CFH (see figure 1). Its C-terminal domains are highly homologous to CFH SCR19–20, enabling binding to similar ligands such as C3b and polyanions. However, lacking regulatory domains, FHR-1 can displace CFH from surfaces without inhibiting complement activation, potentially promoting local amplification. This competitive interaction is enhanced by FHR-1 dimerization.

Dysregulation of the CFH–FHR balance is associated with diseases including Atypical hemolytic uremic syndrome, C3 glomerulopathy (aHUS), and age-related macular degeneration (AMD), highlighting the clinical importance of this regulatory network.

The epitope of antibody clone L20/3 is described to be located within SCR20. This epitope is present in FH and all FHRs. However, FHR-3 is not recognized in BLI analysis. Antibody is, at least but not limited to, applicable in the following applications: ELISA, BLI and western blotting. Please, Contact Hycult Biotech for further information.

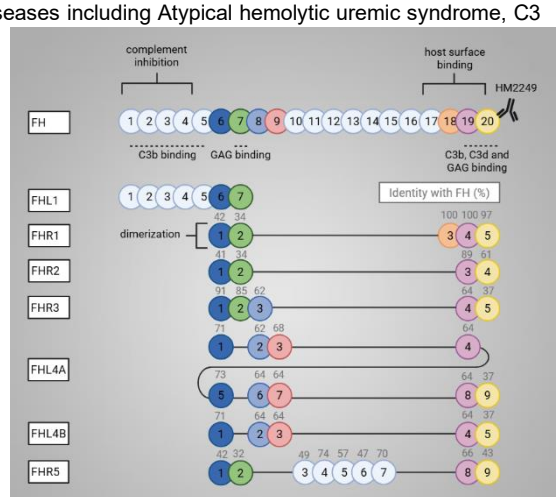


Figure 1: This figure shows a schematic representation of the domain organization of factor H (FH) and the related FH-family proteins. Complement inhibition and C3b binding are primarily located in the N-terminal SCR domains, while the C-terminal region mediates host surface recognition through binding to C3b/C3d and glycosaminoglycans (GAGs). The monoclonal antibody L20/3 is shown to bind to the C-terminal domains of FH. FH-related proteins share varying degrees of sequence identity with FH and display differences in domain composition and dimerization properties.

References

1. Oppermann M. et al; Quantitation of components of the alternative pathway of complement (APC) by enzyme-linked immunosorbent assays. J Immunol Methods 1990, *133*: 181
2. Oppermann M. et al; Elevated plasma levels of the immunosuppressive complement fragment Ba in renal failure. Kidney Int 1991, *40*: 939
3. Oppermann M et al; Assessment of complement activation *in vivo*. Immunopharm 1991, *24*: 119

Storage&stability

Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.

Precautions

For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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Date

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