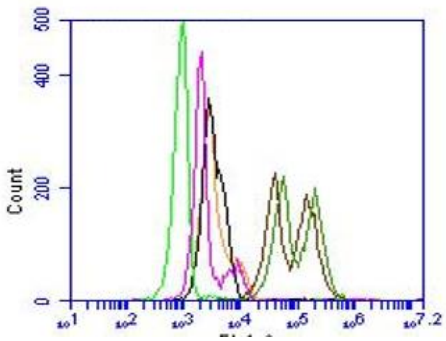
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	Calprotectin, Human, clone 27E10		
Catalog number	HM2156-5MG		
Lot number	-	**Expiry date**	-
Volume	-	**Amount**	5 mg
Formulation	0.2 µm filtered in PBS	**Concentration**	>0.5 mg/ml
Host Species	Mouse IgG1	**Conjugate**	None
Endotoxin	N.A.	**Purification**	Protein G
Storage	4°C		

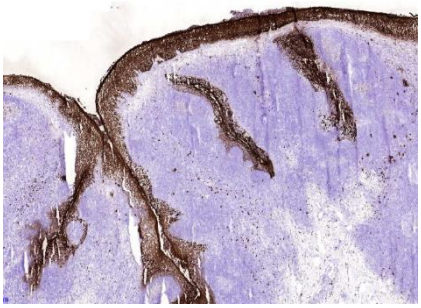
Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	1,3		1,2,4	1		4,8	2,4,5	5,6
Yes	●		●	●		●	●	●
No		●			●			
N.D.								

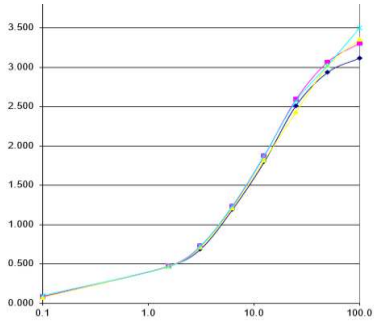
N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot



FC: Flow cytometric detection of Calprotectin with HM2156. The green and black line represent the isotype control in 2 concentrations, the pink line the secondary antibody only and the green and brown line HM2156 in a concentration of 1 and 3 µl/200000 cells.



IHC-F: Staining of squamous epithelia of frozen tonsil section with calprotectin antibody HM2156 (dilution used was 1:100).



IA: HM2156 used as capture antibody. Different concentrations were tested (0.5-2 µg/ml).

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.

- W: non-reduced;~22 kDa; doesn't recognize the single proteins S100A8 and S100A9
- HPLC: reduced (~17 kDa) and non-reduced(various sizes due to association with other elements (Ref1)
- IHC-F: acetone fixation; 0.1 % hydrogen peroxide treatment to reduce enogenous peroxidise activity; positive control: inflammatory tissue; negative control: normal human tissue (skin, lung, colon) (Ref1)
- FC: Extracelullar expression on monocytes, as negative control HL-60, platelets, lymphocytes can be used.
- Positive control: Human granulocytes; Negative control: Platelets, lymphocytes, HL-60 cells.

General Information

Description

The monoclonal antibody 27E10 recognizes an epitope specific for the S100A8/A9 heterocomplex that is not exposed on the individual subunits S100A8 (MRP8, calgranulin-A) or S100A9 (MRP14, calgranulin-B). The calcium-binding migration inhibitory factor-related proteins, MRP-8 (S100A8) and MRP-14 (S100A9) belong to the S100 protein family. The expression of these proteins is largely confined to the cytosol of neutrophils and monocytes. The complex formation of these proteins is a calcium-dependent process. The S100A8/A9 heterocomplex, also called MRP-8/MRP-14 complex or calprotectin, comprises 60% of the cytoplasmic protein fraction of circulating polymorphonuclear granulocytes and is also found in monocytes, macrophages and ileal tissue eosinophils. Peripheral blood monocytes carry the antigen extra- and intracellularly, neutrophils only intracellularly. The S100A8/A9 complex has antibacterial, antifungal, immunomodulating and antiproliferative effects. Besides this it is a potent chemotactic factor for neutrophils.

Plasma concentrations are elevated in diseases associated with increased neutrophil activity, like inflammatory bowel disease. Granulocytes terminate their existence after transmigration through the intestinal wall. Therefore calprotectin is also detectable in feces. Elevated levels of calprotectin have been observed in body fluids such as plasma, saliva, gingival crevicular fluid, stools, and synovial fluid during infection and inflammatory conditions. The monoclonal antibody 27E10 can be used for early detection of inflammatory macrophages, for the characterization of tumorous tissues and the monitoring of peripheral blood cell cultures. The antibody 27E10 does not react with lymphocytes or platelets.

Immunogen Human blood monocytes

Aliases S100A8/A9, MRP-8/MRP-14, calprotectin, calgranulin-A/calgranulin-B, L1-protein

Cross reactivity Mouse: No; Rhesus Monkey: Yes (subpopulation of macrophages).

References

1. Zwadlo, G et al; A monoclonal antibody to a subset of human monocytes found only in the peripheral blood and inflammatory tissues. J Immunol 1986, *137*: 512
2. Hessian, P et al; The heterodimeric complex of MRP-8 (S100A8) and MRP-14 (S100A9) – Antibody recognition, epitope definition and the implications for structure. Eur J Biochem 2001, *268*: 353
3. Kuhn, A et al; Upregulation of epidermal surface molecule expression in primary and ultraviolet-induced lesions of lupus erythematosus tumidus. Br J Dermatol 2002, *146*: 801
4. Champaiboon, C et al; Calprotectin S100A9 calcium-binding loops I and II are essential for keratinocyte resistance to bacterial invasion. J Biol Chem 2009, *284*: 7078
5. Williams, S et al; a novel proinflammatory role for annexin a1 in neutrophil transendothelial migration, Thesis 2009
6. Stork, M et al; Zinc Piracy as a Mechanism of Neisseria meningitidis for Evasion of Nutritional Immunity, PlosOne 2013, *9*:e1003733
7. Ohri, C et al. The Tissue Microlocalisation and Cellular Expression of CD163, VEGF, HLA-DR, iNOS, and MRP 8/14 Is Correlated to Clinical Outcome in NSCLC. PLoS ONE 2011, *6*: 21874
8. Tardif, M et al; Secretion of S100A8, S100A9, and S100A12 by Neutrophils Involves Reactive Oxygen Species and Potassium Efflux. J Immunol Res 2015, *2015*: 296149

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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Approved by Manager of QC	Date
Brenda Teunissen	07/10/2019

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