

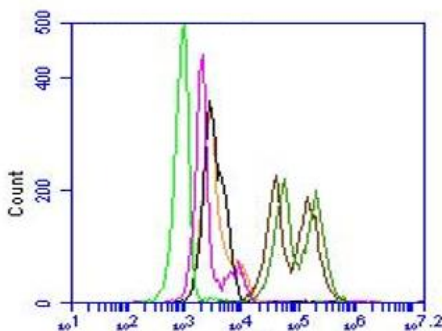
## CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

<b>Product name</b>	Calprotectin, Human, clone 27E10, FITC conjugated		
<b>Catalog number</b>	HM2156F-100UG		
<b>Lot number</b>	-	<b>Expiry date</b>	-
<b>Volume</b>	1 ml	<b>Amount</b>	100 µg
<b>Formulation</b>	0.2 µm filtered in PBS+1%BSA+0.02%Na3	<b>Concentration</b>	100 µg/ml
<b>Host Species</b>	Mouse IgG1	<b>Conjugate</b>	FITC
<b>Endotoxin</b>	N.A.	<b>Purification</b>	Protein G
<b>Storage</b>	4°C		

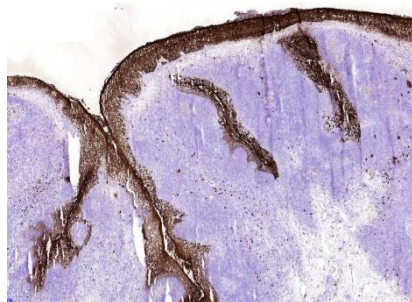
### Application notes

	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.								

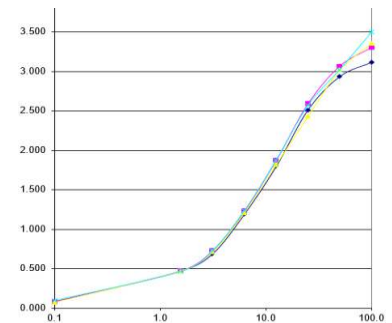
N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot



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 endogenous peroxidase activity; positive control: inflammatory tissue; negative control: normal human tissue (skin, lung, colon) (Ref1)
- FC: Extracellular 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.

<b>Immunogen</b>	Human blood monocytes
<b>Aliases</b>	S100A8/A9, MRP-8/MRP-14, calprotectin, calgranulin-A/calgranulin-B, L1-protein
<b>Cross reactivity</b>	Mouse: No; Rhesus Monkey: Yes (subpopulation of macrophages).
<b>References</b>	<ol style="list-style-type: none"><li>1. Zwadlo, G et al; A monoclonal antibody to a subset of human monocytes found only in the peripheral blood and inflammatory tissues. <i>J Immunol</i> 1986, <i>137</i>: 512</li><li>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. <i>Eur J Biochem</i> 2001, <i>268</i>: 353</li><li>3. Kuhn, A et al; Upregulation of epidermal surface molecule expression in primary and ultraviolet-induced lesions of lupus erythematosus tumidus. <i>Br J Dermatol</i> 2002, <i>146</i>: 801</li><li>4. Champaiboon, C et al; Calprotectin S100A9 calcium-binding loops I and II are essential for keratinocyte resistance to bacterial invasion. <i>J Biol Chem</i> 2009, <i>284</i>: 7078</li><li>5. Williams, S et al; a novel proinflammatory role for annexin a1 in neutrophil transendothelial migration, Thesis 2009</li><li>6. Stork, M et al; Zinc Piracy as a Mechanism of Neisseria meningitidis for Evasion of Nutritional Immunity, <i>PlosOne</i> 2013, <i>9</i>:e1003733</li><li>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. <i>PLoS ONE</i> 2011, <i>6</i>: 21874</li><li>8. Tardif, M et al; Secretion of S100A8, S100A9, and S100A12 by Neutrophils Involves Reactive Oxygen Species and Potassium Efflux. <i>J Immunol Res</i> 2015, <i>2015</i>: 296149</li></ol>
<b>Storage&amp;stability</b>	Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.
<b>Precautions</b>	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.

---

We hereby certify that the above-stated information is correct and that this product has been successfully tested by the Quality Control Department. This product was released for sale according to the existing specifications. This document has been produced electronically and is valid without a signature.

Approved by Manager of QC  
Robbert Zwinkels

Date  
03/12/2019

Do you have any questions or comments regarding this product? Please contact us via [support@hycultbiotech.com](mailto:support@hycultbiotech.com).