



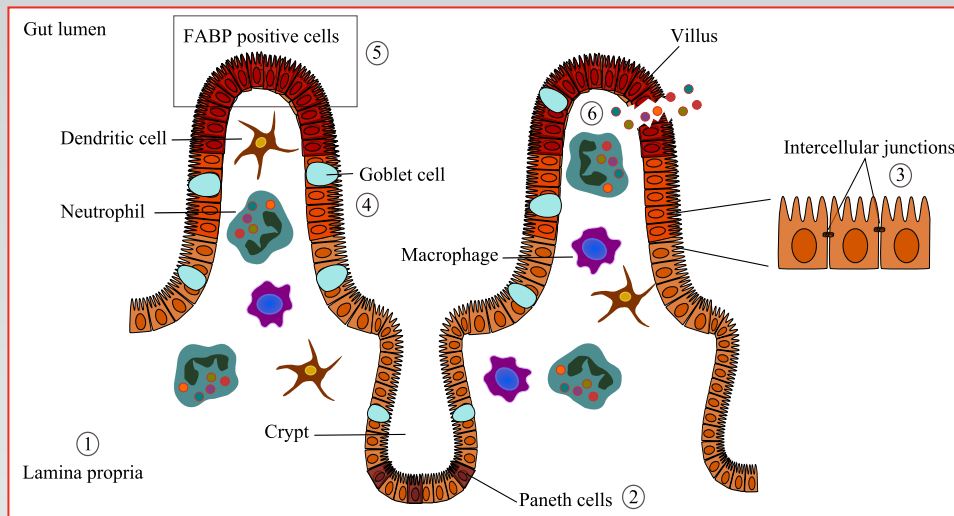
# Hycult Scope



## Intestinal inflammation

Gastrointestinal tissue is constantly under attack by luminal bacteria, viruses and other harmful microbiota. Epithelial cells and the immune cells of mucosal tissues, form an important line of defense. Mucosal damage is pivotal to the onset of intestinal inflammation and the etiology of, for example, inflammatory bowel disease (IBD). It is, therefore, essential to understand the mechanisms underlying mucosal damage.

Epithelial cells form a biological barrier that prevents interaction of luminal pathogens with the immune cells in the lamina propria (1). The crypts of the small gut are protected against microbiota by an array of microbial peptides among which defensins (e.g. HD5), lysozyme and phospholipase A2. These peptides are produced by Paneth cells, specialized secretory epithelial cells present at the bottom of the crypt (2). The physical barrier function of the epithelium consists of the cellular junctions between neighboring epithelial cells (3) and the mucous layer produced by Goblet cells that covers the microvilli of the epithelium (4).



Pathogens breaching the intestinal barrier trigger the innate immune system, followed by a pathogen-specific response provided by the adaptive immune system. Cellular damage to lining epithelium occurs in inflammatory diseases like Crohn's disease and ulcerative colitis, but also in other disease states as colon cancer and intestinal ischemia. This damage leads to release of cytosolic proteins, like the fatty acid binding proteins I-FABP, L-FABP and I-LBP, which can subsequently be detected in serum, plasma, feces and/or urine (5). The regional distribution of these proteins allows localization of the cellular damage. Epithelial damage results in inflammation leading to infiltration of the lamina propria with immune cells like macrophages, neutrophils, and dendritic cells. Activation of neutrophils leads to release of large amounts of granular and cytosolic products (6), like BPI, CD14, LL-37, Elafin/SKALP, myeloperoxidase, lactoferrin, elastase, nGAL and calprotectin. The latter four can be detected in stool after epithelial intestinal damage.

In summary, assessment of specific cellular damage and infection markers can be a valuable and non-invasive tool to study the mechanisms involved in mucosal damage and underlying intestinal inflammation.

## Epithelial and endothelial damage

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### Assays for intestinal research

NEUTROPHIL PRODUCT ELISA		CAT. #	ADHESION MOLECULE ELISA		CAT. #
BPI, Human	Unique	HK314	sICAM-1, Human		HK304
Calprotectin, Human		HK325	sE-Selectin, Human		HK305
Elastase, Human		HK319	sMAdCAM-1, Human	Unique	HK337
Lactoferrin, Human	New	HK329	<b>INTESTINAL DAMAGE MARKER ELISA</b>		
MPO, Human		HK324	I-FABP, Human	Unique	HK406
MPO, Mouse, Rat	Unique	HK210	I-LBP (IL-FABP), Mouse, Rat	Unique	HK409
nGAL, Human	New	HK330	L-FABP, Human	Unique	HK404
			L-FABP, Mouse, Rat	Unique	HK405
			L-FABP, Swine	Unique	HK408

## Hot news in intestinal research:

### NOD2/CARD:

Mutations in this protein, involved in bacterial recognition by cells involved in innate immunity, is one of the risk factors for developing IBD.

Hugot J-P et al; Am J Gastroenterol 2007, 102: 1

### Finding NEMO:

Epithelial NEMO (also called I $\kappa$ B kinase-c (IKKc)) links innate immunity to chronic intestinal inflammation.

Nenci A et al; Nature 2007, 446: 557

### Tropomyosin 5:

In ulcerative colitis, autoantibodies against human tropomyosin isoform 5 destroy colonic epithelial cells through antibody and complement-mediated lysis.

Ebert E et al; Cell Immunol 2006, 244: 43

## Non-invasive markers for intestinal diseases

Inflammatory bowel disease (IBD) is, on the basis of its clinical features and histopathology, often grouped into two major entities, ulcerative colitis (UC) and Crohn's disease (CD). A hallmark in chronically inflamed intestine is hyper adhesiveness of the local microvasculature. This results in migration of immune cells, including neutrophils, into the infected mucosal site. As a consequence of IBD, the gastrointestinal mucosal epithelial lining is damaged. In line, neutrophil accumulation in the intestinal lumen was found to correlate with clinical disease activity and epithelial injury. Instead of invasive procedures, quantification of inflammatory parameters in feces appears to be an attractive approach to discriminate between patients with mild and severe intestinal inflammation and for follow-up of patients with chronic intermittent inflammation. Neutrophil antimicrobial proteins, like Calprotectin, Lactoferrin, Elafin/SKALP, and MPO as well as epithelial and endothelial adhesion molecules, such as sMAdCAM-1 have been shown to be promising non-invasive biomarkers for the assessment of intestinal inflammation.

- sMAdCAM-1 is upregulated during intestinal inflammation<sup>1</sup>.
- Fecal PMN-elastase and Calprotectin support the differentiation of chronic IBD from IBS and correlate with the severity of inflammation<sup>2</sup>.
- Fecal Calprotectin and Lactoferrin are sensitive and specific markers to differentiate IBD from IBS<sup>3</sup>.
- Elafin/SKALP-1 is upregulated in patients with UC<sup>4</sup>.
- Lactoferrin and Calprotectin determination reflects endoscopic and histological disease activity in UC<sup>5</sup>.
- Lactoferrin and Myeloperoxidase (MPO) are excellent correlative markers for evaluating the disease activity of CD<sup>6</sup>.
- Plasma intestinal fatty acid binding protein (I-FABP) concentrations increase following intestinal ischemia<sup>7</sup>.
- I-FABP is a sensitive marker for ischemia in mechanical small bowel obstruction<sup>8</sup>.
- I-FABP may serve as a diagnostic marker for early intestinal mucosal compromise<sup>9</sup>.

#### Selected articles:

1. Bachmann, C et al; Gastroenter 2006, 130: 8
2. Silberer, H et al; Clin Lab 2005, 51: 117
3. Lundberg, J et al; Nat Clin Pract Gastroenterol Hepatol 2005, 2: 96
4. Lawrance, I et al; Inflamm Bowel Dis 2001, 7: 226
5. D'Inca, R et al; Int J Colorectal Dis 2007, 22: 429
6. Kayazawa, M et al; Am J Gastroenterol 2002, 97: 360
7. Gollin, G et al; Surgery 1993, 113: 545
8. Cronk, D et al; Curr Surg 2006, 63: 322
9. Lieberman, J et al; Surgery 1997, 121: 335

## Stop exercising and dieting!

Overweight? It's not laziness and you're not eating too much. It is those nasty gut bugs that may cause overweight! Researchers at Washington University School of Medicine found that intestinal bacteria may contribute to obesity.

Ley R et al; Nature 2006, 444: 1022  
Backhed F et al; PNAS 2007, 104: 979

## Hot in research

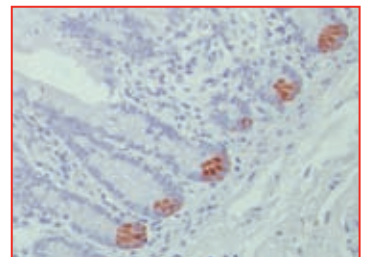
### Human $\alpha$ -defensin 5 expression in intestinal metaplasia of the upper gastrointestinal tract.

Human  $\alpha$ -defensin 5 (HD5) is predominantly expressed in Paneth cell (PC) granules in the crypts of Lieberkühn in the small intestine. HD5 is stored as propeptide and released as mature peptide after PC trypsin digestion to exert extracellularly its antibiotic functions. Interestingly, in metaplasia of the upper gastrointestinal tract, anti-HD5 antibody allows immunohistochemical detection of PCs, even when routine histology fails to demonstrate their characteristic cytoplasmic granules. Therefore, HD5 immunostaining is potentially useful for analysis of intestinal metaplasia of the upper gastrointestinal tract, as well as for detection of PC metaplasia in general.

Shen, B et al; J Clin Pathol 2005, 58: 687

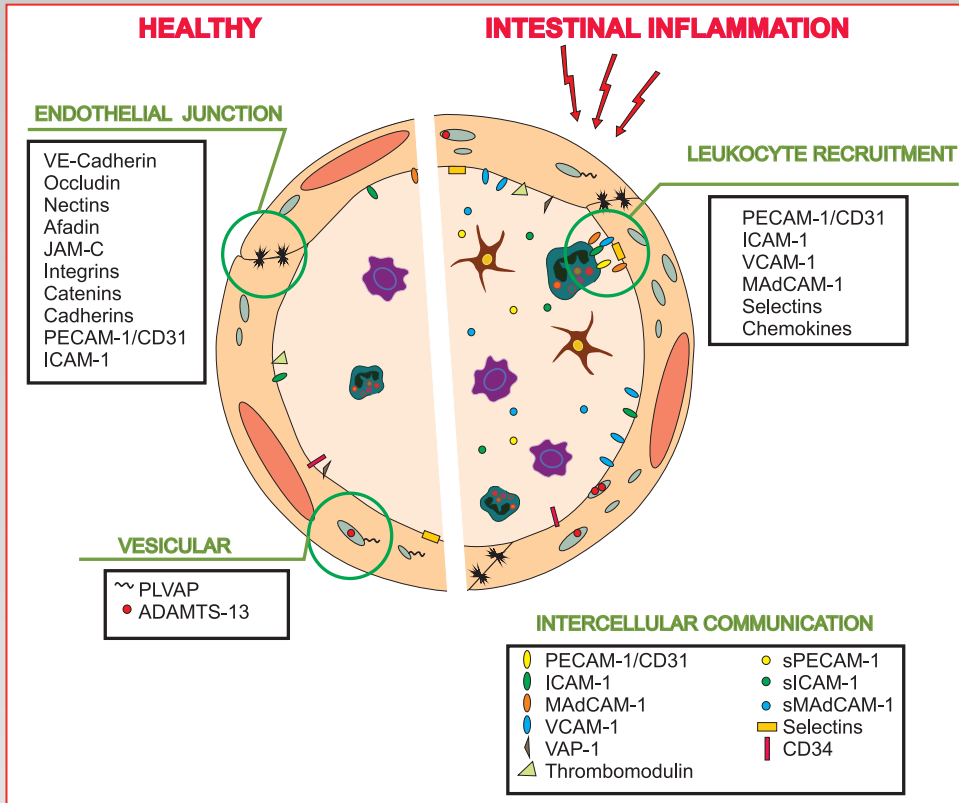
#### ANTIBODIES:

ANTIBODIES:	CAT. #
$\alpha$ -Defensin 5 (HD5), Human mAb 8C8	<b>Unique</b> HM2228
$\alpha$ -Defensin 5, Rabbit mAb R3	<b>Unique</b> HM4008



HD5 expression in crypts. Staining with antibody to HD5 (mAb 8C8, Cat. # HM2228).

## Endothelial cell communication



## Intestinal epithelial BPI

An important facet of mucosal innate immunity.

GO Canny, Ph.D. Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Studies in recent years have focused much attention on antimicrobial proteins and peptides as common components of both epithelial and phagocytic innate host defenses. Intestinal epithelial cells express a variety of antimicrobial peptides and proteins, like the endotoxin-neutralizing protein bactericidal permeability-increasing protein (BPI). BPI binds with high affinity to the lipid A portion of endotoxin. The N-terminal half of the molecule is important for the antibacterial and endotoxin neutralization functions. The C-terminal half contains the motif necessary for bacterial opsonisation. BPI is cytotoxic to a wide range of Gram negative bacterial species including encapsulated, serum resistant E.coli K1-R, Salmonella and Shigella species. BPI is expressed on the cell surface and more recent studies have shown it is not released from intestinal epithelial cells in vitro and appears to also have a cytoplasmic distribution. Furthermore, BPI plays a role in dampening responses of epithelia to endotoxin. A cell line overexpressing BPI possessed an increased ability to kill Salmonella typhimurium and displayed attenuated release of the bacterially-induced chemokine IL-8.

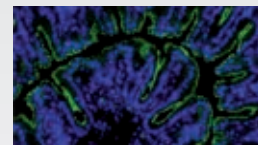
### BPI AND OTHER ANTIMICROBIAL PRODUCTS:

Specificity	Cat. #
BPI, Human, ELISA	<b>Unique</b> HK314
BPI, Human, mAb 3F9	<b>Unique</b> HM2041
BPI, Human, mAb 4H5	<b>Unique</b> HM2042
BPI, Human, mAb 4E3	<b>Unique</b> HM2170
BPI, Human, pAb	<b>Unique</b> HP9022
$\alpha$ -Defensins 1-3, Human, ELISA	<b>Unique</b> HK317
Elafin/SKALP, Human, ELISA	<b>Unique</b> HK318
LBP, Human, ELISA	HK315
LBP, Mouse, ELISA	<b>Unique</b> HK205
LBP of various species, ELISA	<b>Unique</b> HK503
LL-37, Human, ELISA	<b>Unique</b> HK321
sCD14, Human, ELISA	HK320
SLPI, Human, ELISA	HK316

In summary, BPI is involved in bacterial killing by intestinal epithelial cells and also down regulates the response of epithelial cells to LPS. Endogenous mechanism(s) to diminish aberrant activation of epithelial cells may be the primary function of BPI. It is likely that the inhibition of LPS signaling is a very important aspect of BPI expression. Clearly, BPI is a potentially significant component of innate defense at mucosal surfaces.

### ENDOTHELIAL AND EPITHELIAL ANTIBODIES

Specificity	Cat. #
ADAMTS-13, Human, mAb 5C11	<b>New</b> HM2225
ADAMTS-13, Human, mAb 20A5	<b>New</b> HM2226
$\alpha$ -catenin, Human, mAb 1G5	HM2118
$\alpha$ V $\beta$ 3-Integrin, Human, mAb BV3	HM2034
$\beta$ 3-Integrin subunit, Human, mAb BV4	HM2035
$\beta$ -Catenin, Human, mAb 9F2	HM2112
$\beta$ -Integrin, Human, mAb BV7	HM2033
CD31, PECAM-1, Human, mAb BV8	HM2039
CD31, PECAM-1, Mouse, mAb MEC7.46	HM1013
CD31, PECAM-1, Mouse, mAb ER-MP12	HM1084
CD34, Mouse, mAb MEC14.7	HM1015
CD36, Human, mAb FA6-152	HM2122
CD36, Mouse, mAb CRF D-2712	HM1074
CD36, Rat, mAb UA009	<b>Unique</b> HM3019
CD73, Human, mAb 4G4	<b>New</b> HM2215
CD141, Thrombomodulin, Human, mAb RTM96	HM2146
CD141, Thrombomodulin, Human, mAb RTM98	HM2147
CD154, CD40L, Human, mAb 24-31	<b>New</b> HM2222
EMAP II, Human, mAb 546-2	HM2185
Endoglin, CD105, Human, mAb E9	HM2140
Endostatin, Human, mAb 1837-46	HM2188
Endothelial cell antigen (RECA), Rat, mAb RECA1	HM3012
Endothelial Protein C Receptor (EPCR), Human, mAb RCR-379	<b>Unique</b> HM2144
Endothelial Protein C Receptor (EPCR), Human, mAb RCR-252	<b>Unique</b> HM2145
E-Selectin, CD62-E, Human, mAb ENA1	HM4001
E-Selectin, CD62-E, Human, pAb	HP9017
ICAM-1, CD54, Human, mAb HM1	HM4004
ICAM-1, CD54, Human, pAb	HP9018
JAM-C, Mouse, Human, mAb CRAM-19 H36	HM1056
JAM-C, Mouse, Human, mAb CRAM-18 F26	HM1057
L-Afadin, Rat, Human, Mouse, mAb 3	<b>Unique</b> HM3013
MAdCAM-1, Human, mAb 314G8	HM2207
Nectin-2, Mouse, Human, mAb 502-57	HM1052
Nectin-3, Mouse, mAb 103-A1	<b>Unique</b> HM1053
Occludin, Human, Mouse, Rat, pAb	<b>New</b> HP9047
PAI-1, Human, mAb MA-33HF7	HM2179
PAI-1, Human, mAb MA-55F4C12	HM2180
PAI-1, Human, mAb MA-56A7C10	HM2181
PAI-1, Rat, mAb MA-124K1	HM3026
PLVAP, Human, mAb 174/2	<b>Unique</b> HM2214
VAP-1, Human, Rat, mAb 174-5	<b>Unique</b> HM2213
VAP-1, Mouse, mAb 7-88	<b>Unique</b> HM1094
VCAM-1, CD106, Human	HM4006
VE-Cadherin, Human, mAb 1G11B1	HM2032
VG5Q, Human, pAb	HP9037
VG5Q, Mouse, pAb	HP8017



ZO-1 in mouse colon. Immunofluorescence staining with antibody to ZO-1 (pAb, Cat.# HP9043).

Specificity	Cat. #
Barmotin/7H6 antigen, Human, mAb 7H6	<b>Unique</b> HM2102
$\alpha$ -Defensin-5, Human, mAb 8C8	<b>Unique</b> HM2228
Desmoglein-1, Human, mAb 27B2	HM2113
Desmoglein-2, Human, mAb 6D8	HM2114
Desmoglein-3, Human, mAb 5G11	HM2115
Elafin/SKALP, Human, mAb TRAB2O	<b>Unique</b> HM2062
Elafin/SKALP, Human, mAb TRAB2F	<b>Unique</b> HM2063
Elafin/SKALP, Human, pAb	HP9025
Galectin-3, Human, Mouse, mAb B2C10	HM2186
JAM-A, Human, mAb BV16	HM2098
JAM-A, Human, mAb M.Ab.F11	HM2099
JAM-A, dom. 1, Human, pAb	<b>Unique</b> HP9041
JAM-A, dom. 2, Human, pAb	<b>Unique</b> HP9042
JAM-A, Mouse, mAb BV12	<b>Unique</b> HM2099
Plakoglobin, Human, mAb 15F11	HM2116
Vitronectin, Human, mAb BV1	HM2036
ZO-1, Human, Mouse, pAb	<b>New</b> HP9043
ZO-1 $\alpha$ +, Human, Mouse, Rat, pAb	<b>New</b> HP9044
ZO-1 $\alpha$ -, Human, Mouse, Rat, pAb	<b>New</b> HP9045
ZO-2, Human, Mouse, pAb	<b>New</b> HP9046



## New assay for L-Ficolin quantification

### Human L-Ficolin ELISA:

L-Ficolin recognizes distinct danger-associated molecular patterns (DAMP) like GlcNAc-structures in LTA and fungal 1,3-β-D-glucan. L-Ficolin also recognizes N-acetylated carbohydrates and other non-carbohydrate acetylated compounds such as acetylcholine.

Furthermore, L-Ficolin recognizes apoptotic cells and participates in the removal of host cells. L-ficolin circulates in complex with MASP-2 and can activate the lectin pathway.

Low serum levels of L-Ficolin are reported to be associated with recurrent respiratory infections in children. Interestingly, L-Ficolin has been implicated in the unique immune challenge during pregnancy. In maternal plasma of normal pregnancies a 4- to 5-fold increase in L-Ficolin was detected compared to healthy non-pregnant persons. However, significantly lower L-Ficolin maternal plasma concentrations were associated with pre-eclamptic pregnancies. Therefore, assessment of L-Ficolin is warranted to study its regulatory role in the innate immune system.

### Now available:

ELISA		CAT. #
L-Ficolin, Human	Unique	HK336
Functional MBL/MASP-2, Human, (C4 deposition)	Unique	HK327
MASP-2, Human	Unique	HK326
MBL, Human, (lectin activity)		HK323
SP-D, Human		HK335
TCC/sC5b-9/MAC, Human		HK328

### Also available:

- ▶ Antibodies to human MBL, MASP-1, MASP-2, MASP-3, L-Ficolin, H-Ficolin and M-Ficolin.

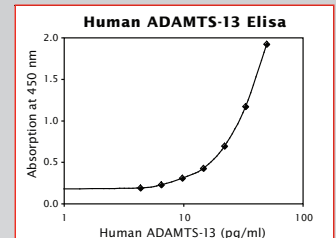
## ADAMTS-13: VWF cleaving protease

### New assay for ADAMTS-13 quantification

ADAMTS-13 (A Disintegrin And Metallo-protease with Thrombospondin type 1 domain-13) is a zinc-containing metallo-protease produced by hepatic stellate cells and in smaller amounts by human endothelial cells. ADAMTS-13 is also known as Von Willebrand Factor (VWF) cleaving protease. VWF plays an important role in the regulation of platelet adhesion at damaged sites. Low or even absent enzyme activity

of ADAMTS-13 results in unusually large VWF multimers present in plasma of patients suffering from thrombotic thrombocytopenic purpura (TTP). This life-threatening coagulation disorder can lead to ischemic disease with (multiple) organ failure. Low levels of ADAMTS-13 are found in stroke, liver cirrhosis, renal failure and sepsis.

ADAMTS-13	
Reagent	Cat. #
ADAMTS-13, Human, ELISA	HK338
ADAMTS-13, Human, mAb 5C11	HM2225
ADAMTS-13, Human, mAb 20A5	HM2226



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## Hot new products

### Antibodies to VAP-1 and PLVAP for vascular research

#### Vascular Adhesion Protein-1 (VAP-1)

- VAP-1 is present in endothelial cells, smooth muscle cells, adipocytes, and in follicular dendritic cells.
- Mediates leukocyte attraction to sites of inflammation.
- Inhibits lymphocyte infiltration in liver allografts.

#### PLVAP (PAL-E, PV1, FELS)

- Unique vascular staining pattern of endothelial cell antigen.
- Specifically up-regulated in endothelium of brain tumors.
- Expressed in the vasculature of most other tumors.



Vascular staining of VAP-1 in mouse spleen. Immunohistochemical staining with antibody to VAP-1 of a frozen section (mAb 7-88, Cat.# HM1094).

ANTIBODIES		CAT. #
PLVAP, Human, mAb 174/2	Unique	HM2214
VAP-1, Human, Rat, mAb 174/5	Unique	HM2213
VAP-1, Mouse, mAb 7-88	Unique	HM1094

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