Advanced Cell Diagnostics

Specific Detection of Inflammation Markers with Tissue Context Using RNAscope® Technology

Detect your target of interest with ease

- High Sensitivity – single molecule detection
- High Specificity – specific target detection
- Detection of virtually ANY gene in ANY genome in ANY tissues
- Robust Methodology and Ease of Use – automated assays and convenient manual assays

RNASCOPE® DELIVERS

MD+MC

IN A SINGLE ASSAY

Molecular Detection visualizes and quantifies expressed genes specifically and with single-copy detection.

Morphological Context localizes expressed genes at single-cell resolution in tissue context.

Detection of Immune Biomarkers in the Tissue

Understanding molecular mechanisms is critical for identifying pathogenesis, evaluating disease progression and developing new therapeutic strategies for autoimmunity, infectious diseases, and cancer. RNAscope® *in situ* hybridization (ISH) technology enables precise cell- and tissue-specific localization of RNA transcripts. With RNAscope® ISH assays, you can detect specifically expressed immune markers in lymphoid and non-lymphoid tissues while maintaining the morphological context. For example, RNAscope® chromogenic *in situ* hybridization (CISH) robustly identified the expression pattern of *CD274* mRNA in formalin-fixed paraffin-embedded (FFPE) kidney cancer tissue (Figure 1).

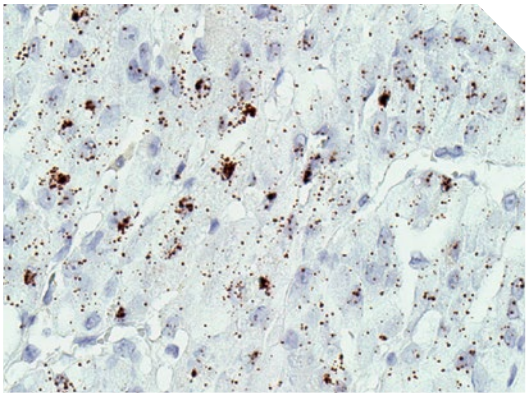

FIGURE 1. *CD274* mRNA expression in a FFPE human kidney tumor tissue using the RNAscope® 2.0 HD Reagent Kit -BROWN. Each brown spot represents a single *CD274* mRNA.

Visualization of any RNA

In the past few decades, the field of immunology benefited greatly from emergent immunoassays, flow cytometry and immunohistochemistry. However, these assays are limited by the availability and specificity of antibodies. With RNAscope® ISH technology, one can assess the expression of any genes in any species. RNAscope® ISH is particularly powerful in detecting mRNA of low-expressing or secreted proteins (Figure 2), as well as targets to which specific antibodies are not available.

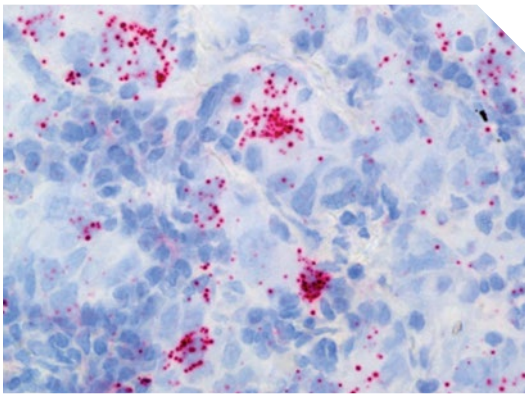

FIGURE 2. *IL6* mRNA expression in FFPE human lung cancer tissue using the RNAscope®2.0 HD Reagent Kit –RED. Each red spot represents a single *IL6* mRNA.

Specific RNA Localization in Tissue

Sensitive and Specific Molecular Detection

RNAscope® ISH technology achieves high specificity and sensitivity in detection of gene expression. Signal amplification coupled with simultaneous background suppression strategy results in single-RNA-molecule detection even in partially degraded samples (Figure 3). Unique double Z probes design eliminates cross hybridization to unintended targets and routinely distinguishes RNA sequences with up to 85% homology.

Twenty double Z probe pairs are designed per target allowing detection of intact or partially degraded RNA molecule.

Ease of Use and Robustness

Extensive assay development was done to ensure that the RNAscope® assay will work with a wide range of tissue types—both FFPE and fresh frozen are routinely used. The RNAscope® ISH workflow is similar to IHC and can be automated. Thousands of off-the-shelf catalog probes and several probe pools are already available. Custom probes can be designed and manufactured in less than three weeks.

Detection of *IL17RA*+ Neurons in Spinal Cords by Highly Sensitive RNAscope® ISH

Reported by: Meng *et al.*, 2014

Inflammatory cytokine Interleukin-17 (*IL-17*) is expressed in the central nervous system and involved in the pathogenesis of several autoimmune diseases including multiple sclerosis, rheumatoid arthritis and psoriasis. Recently, Meng *et al.* investigated the role of *IL-17* in inflammatory pain by studying a rat induced-hyperralgesia model, and showed that *IL-17* in spinal cord enhanced pain. In their study, the researchers measured the expression pattern of *IL-17* and its receptor *IL17RA* in rat FFPE tissues. RNAscope® CISH showed that *IL17RA* was expressed by oval and round cells at ~7 um in rat spinal cord (Figure 4). Other supporting data suggested these cells are neurons. This study suggests that *IL17/IL17RA* signaling is involved in the transmission of inflammatory pain in the spinal cord.

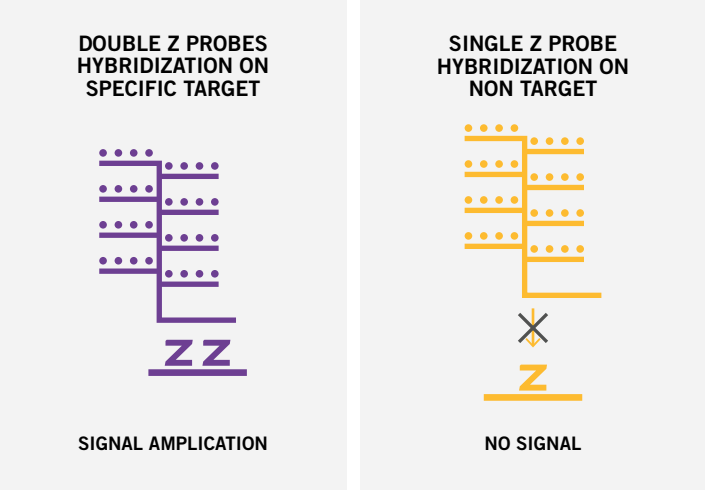

FIGURE 3. In order to substantially improve signal-to-noise ratio of RNA ISH, RNAscope® employs a probe design strategy, in which two independent probes (double Z probes) have to hybridize to the target sequence in tandem in order for signal amplification to occur. Because it is highly unlikely that two independent probes will hybridize to a nonspecific target right next to each other, this design concept ensures selective amplification of target-specific signals.

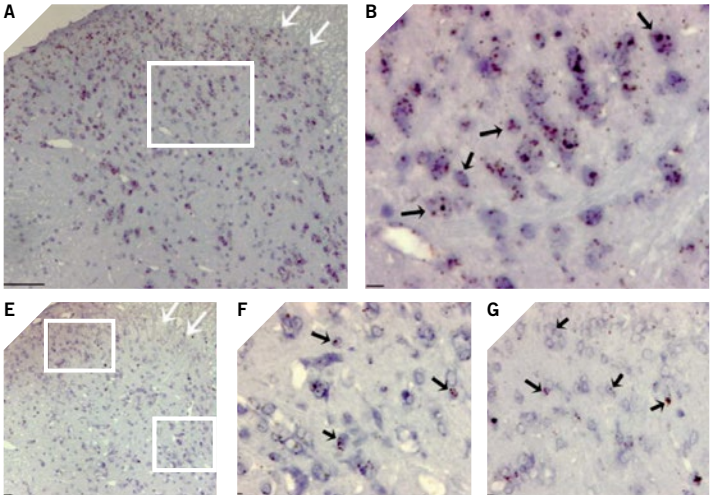

FIGURE 4. Representative microphotographs of RNAscope® staining of the spinal cord. (A) Positive staining of spinal cord using RNAscope® positive control probe, *POLR2A*. (B) Magnification of white box in A; arrows point to cells containing brown, punctate dots in the cytoplasm. (E) RNAscope staining of spinal cord with *IL-17RA* probe. (F and G) Respective magnifications of bottom-right and upper-left boxes in E; arrows point to cells containing brown, punctate dots in the cytoplasm, which shows that *IL-17RA*-specific mRNA was in those cells. The counterstaining demonstrates that the *IL-17RA* mRNA-positive cells are oval and round at~7 um. White arrows point to superficial laminae of the dorsal horn in A and E. Figure from Meng *et al.,* (2013).

Specific and Quantitative RNA Detection by RNAscope® Technology

Validation of IHC Specificity by RNAscope® ISH
Reported by: Østvik, *et al*., 2014

Complement pathway has been suggested to be involved in the pathogenesis of inflammatory bowel disease. However, the local sources of complement factors have not been identified in the inflamed gastrointestinal tract. Østivik *et al* utilized RNAscope® ISH and immunohistochemical staining (IHC) to detect complement factor B mRNA (*CFB*) and protein (fB) in biopsies of active Crohn's Disease (CD) colon (Figure 5). *CFB* mRNA and fB proteins were localized in the luminal epithelial cells, while the epithelium of Lieberkuhn's crypts showed little or no expression of *CFB*.

It is often difficult to ascertain the cellular source(s) of a secreted protein by IHC staining. In this study, the results of RNAscope® ISH identified colonic epithelial cells as the major source of CFB synthesis.

Identification of Expression Pattern with Morphological Context by RNAscope® ISH
Case study: Brenna, *et al*., 2013

Trinitrobenzene sulphonic acid (*TNBS*)-induced colitis is a widely used model for inflammatory bowel disease (IBD) in the study of pathogenesis, cellular mechanisms and potential therapeutic strategies. Brenna *et al*. systematically characterized *TNBS*-induced colitis in rat models and compared the transcription pattern of rat and human IBD colon mucosal tissues. RNAscope® ISH was applied to validate the findings by microarray analysis. The expression of *TLR2* and *TLR4* was significantly up-regulated both in human IBD and TNBS-induced colitis (Figure 6). The *in situ* hybridization results clearly demonstrate the upregulation of *TLR2* and *TLR4* in colon epithelial cells after *TNBS*-administration. RNAscope® ISH assays validated the microarray finding and further delineated the source of inflammatory cytokines in tissue context.

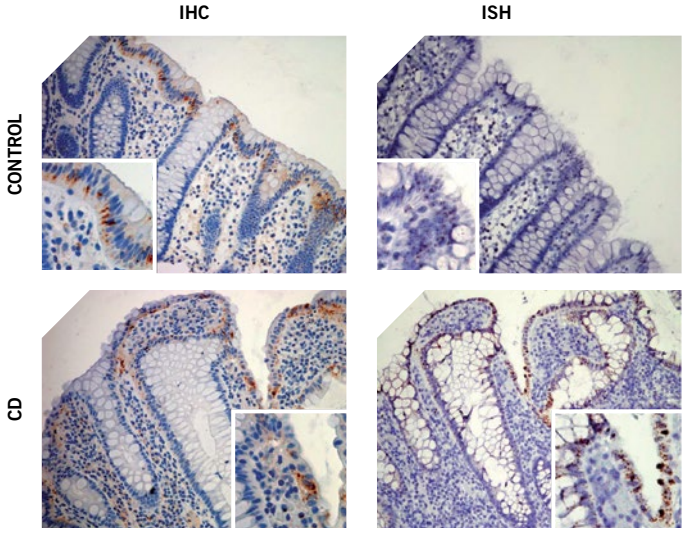

FIGURE 5: IHC and RNAscope® ISH showing complement factor B protein (fB) and mRNA *(CFB)* expression in colonic biopsies in active CD and healthy controls. Serial sections from same biopsy were used to localize protein and mRNA. RNAscope® and IHC staining indicate that both CFB mRNA and factor B protein are localized in the luminal epithelial cells with little or no staining of the epithelium of Lieberkuhn's crypts in colonic mucosa. Original magnifications, ×20 and ×40 (insets). Figure from Østvik, *et al.,* (2014).

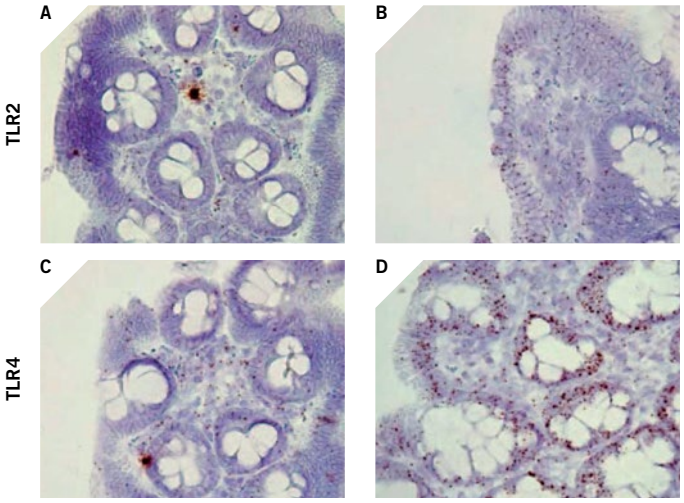

FIGURE 6. RNAscope® *in situ* hybridization (ISH) of *TLR2* and *TLR4* in TNBS colitis mucosal biopsies. (A and C) The scattering expression of *TLR2* and *TLR4* in the epithelium and submucosal immune cells at day 0. (B and D) Increased *TLR2* and *TLR4* expression in the epithelium at day seven of TNBS administration. Figures from Brenna, *et al.*, (2013).

Inflammation Marker Catalog Pooled Probes

A broad spectrum of probe selection is available for inflammation marker analysis. Note each probe vial is sufficient for 20 slides.

CAT No.	Probe Name	CAT No.	Probe Name	CAT No.	Probe Name
521761	Probe - Hs-AICDA	602021	Probe - Hs-PDCD1	402071	Probe - Hs-IL12B
316271	Probe - Hs-CD274-sense	548451	Probe - Hs-PECAM1	586241	Probe - Hs-IL13
600861	Probe - Hs-CD274	542701	Probe - Hs-PLAUR	310931	Probe - Hs-IL17A
578471	Probe - Hs-CD40	605331	Probe - Hs-PROCR	310941	Probe - Hs-IL17F
542341	Probe - Hs-CD40LG	310121	Probe - Hs-PTEN	400301	Probe - Hs-IL18
311271	Probe - Hs-CD44	606051	Probe - Hs-GPR44	556791	Probe - Hs-IL1A
311401	Probe - Hs-CDKN1A	313761	Probe - Hs-PTGDR2-CDS	310361	Probe - Hs-IL1B
603291	Probe - Hs-CFTR	406771	Probe - Hs-PTGER4	603491	Probe - Hs-IL1RL1
556371	Probe - Hs-CHN1	406811	Probe - Hs-PTGS1	401251	Probe - Hs-IL21
400231	Probe - Hs-CLEC4C	406801	Probe - Hs-PTGS2	560811	Probe - Hs-IL22
596021	Probe - Hs-CNR2	407641	Probe - Hs-PYCARD	562851	Probe - Hs-IL23A
313981	Probe - Hs-CSF2	310971	Probe - S100B	406891	Probe - Hs-IL23R-sense
554341	Probe - Hs-CTLA4	555961	Probe - Hs-SERPINE1	406881	Probe - Hs-IL23A-sense
311731	Probe - Hs-CTNNB1	400751	Probe - Hs-DEFB4A	400111	Probe - Hs-IL33
311851	Probe - Hs-CXCL10	319461	Probe - Hs-EGFR-delta	315191	Probe - Hs-IL4
311321	Probe - Hs-CXCL13	310061	Probe - Hs-EGFR	310371	Probe - Hs-IL6
539251	Probe - Hs-CXCR3	528991	Probe - Hs-EPHB3	557201	Probe - Hs-IL6R
310511	Probe - Hs-CXCR4	319861	Probe - Hs-F2RL1	522691	Probe - Hs-IRAK1
594511	Probe - Hs-ANGPT2	407611	Probe - Hs-F3	555091	Probe - Hs-ITGAM
314311	Probe - Hs-APOA1	555921	Probe - Hs-FCGR2A	403541	Probe - Hs-TSLP
591721	Probe - Hs-BCL2	538701	Probe - Hs-FCGR2B	311351	Probe - Hs-VEGF
549171	Probe - Hs-CCL5	560701	Probe - Hs-FLT1	560461	Probe - Hs-VWF
406901	Probe - Hs-CCR1-sense	310311	Probe - Hs-FN1	310421	Probe - Hs-TNF-a
401881	Probe - Hs-CCR1	603131	Probe - Hs-GATA6	605681	Probe - Hs-SNCA
601501	Probe - Hs-CCR5	600301	Probe - Hs-GDF15	310711	Probe - TAC1
559441	Probe - Hs-LCN2	310761	Probe - Hs-HGF	310701	Probe - Hs-TACR1
310461	Probe - Hs-LTA	314331	Probe - Hs-HIF1A-3UTR	400881	Probe - Hs-TGFB1
530491	Probe - Hs-MGST3	605221	Probe - Hs-HIF1A	407121	Probe - Hs-TGFB1-long
311751	Probe - Hs-MMP2	319851	Probe - Hs-HMOX1	403111	Probe - Hs-TLR2
311331	Probe - Hs-MMP9	402951	Probe - Hs-ICAM1	605951	Probe - Hs-TLR3
313811	Probe - Hs-MMP9-30LE	602681	Probe - Hs-IDO1	311281	Probe - Hs-TLR4
603091	Probe - Hs-MPO	559781	Probe - Hs-IFNG	605201	Probe - Hs-TP53
310391	Probe - Hs-MUC1	310501	Probe - Hs-IFN-G	400611	Probe - Hs-TNFRSF1A
312891	Probe - Hs-MUC5AC	314641	Probe - Hs-IGF1-tv1	406971	Probe - Hs-TNFSF13B
599311	Probe - Hs-NAMPT	313031	Probe - Hs-IGF1	402231	Probe - Hs-TNFSF14
549451	Probe - Hs-NGF	602051	Probe - Hs-IL10	403121	Probe - Hs-TNFSF15
554091	Probe - Hs-P2RX7	316061	Probe - Hs-IL10-sense	311951	Probe - Hs-TRPA1
316281	Probe - Hs-PDCD1-sense	402061	Probe - Hs-IL12A	408121	Probe - Hs-CHI3L1

CAT No.	Probe Name	CAT No.	Probe Name	CAT No.	Probe Name
408511	Probe - Hs-PTEN-3UTR	425481	Probe - Hs-FN1-E33	409611-C2	Probe - Hs-CCL20-C2
409041	Probe - Hs-BDNF-CDS	425281	Probe - Hs-IL11	418801-C2	Probe - Hs-CD14-C2
409021	Probe - Hs-SNCA-CDS	425101	Probe - Hs-LTF	417061-C2	Probe - Hs-CD163-C2
410241	Probe - Hs-CALCA	424721	Probe - Hs-NR3C1	311731-C2	Probe - Hs-CTNNB1-C2
409611	Probe - Hs-CCL20	425271	Probe - Hs-S100A8	400751-C2	Probe - Hs-DEFB4A-C2
411261	Probe - Hs-CX3CL1	430701	Probe - Hs-C3	418471-C2	Probe - Hs-FOXP3-C2
411251	Probe - Hs-CX3CR1	428731	Probe - Hs-CFH	402061-C2	Probe - Hs-IL12A-C2
412601	Probe - Hs-DPP4-CDS-XMmu	428381	Probe - Hs-EGFR-tv4	402071-C2	Probe - Hs-IL12B-C2
411911	Probe - Hs-HAMP	427811	Probe - Hs-GAS6	310941-C2	Probe - Hs-IL17F-C2
412641	Probe - Hs-MMP1	428351	Probe - Hs-HLA-DRB1	401251-C2	Probe - Hs-IL21-C2
413471	Probe - Hs-NOD1	426691	Probe - Hs-HLA-G	560811-C2	Probe - Hs-IL22-C2
413481	Probe - Hs-NOD2	319391	Probe - Hs-IL5	562851-C2	Probe - Hs-IL23A-C2
410141	Probe - Hs-PRTN3	429811	Probe - Hs-PARP1	419741-C2	Probe - Hs-NFE2L2-C2
412291	Probe - Hs-TNFRSF11B	417581	Probe - Hs-S100A9	413471-C2	Probe - Hs-NOD1-C2
411841	Probe - Hs-TNFSF10	429781	Probe - Hs-SIRT1	413481-C2	Probe - Hs-NOD2-C2
412271	Probe - Hs-TNFSF11	427311	Probe - Hs-SOD1	417581-C2	Probe - Hs-S100A9-C2
412011	Probe - Hs-VEGFA-noXrodent	425631	Probe - Hs-STAT3	416961-C2	Probe - Hs-SDC1-C2
416041	Probe - Hs-SPHK1	426481	Probe - Hs-TNFAIP3	400611-C2	Probe - Hs-TNFRSF1A-C2
415381	Probe - Hs-TRPV1	430601	Probe - Hs-TNFRSF11B-O1	417651-C2	Probe - Hs-TNFRSF1B-C2
419211	Probe - Hs-AKT1	430591	Probe - Hs-TNFSF11-O1	424991-C2	Probe - Hs-NOS2-C2
418321	Probe - Hs-APP	311851-C2	Probe - Hs-CXCL10-C2	311271-C2	Probe - Hs-CD44-C2
423811	Probe - Hs-CCL2	605551-C2	Probe - Hs-CALCA-C2	427151-C2	Probe - Hs-CXCL1-C2
417061	Probe - Hs-CD163	403421-C2	Probe - Hs-MMP3-C2	427031-C2	Probe - Hs-FAS-C2
422991	Probe - Hs-CXCL12	311331-C2	Probe - Hs-MMP9-C2	426581-C2	Probe - Hs-THBS1-C2
418731	Probe - Hs-EGFR-no-X-Mm	310391-C2	Probe - Hs-MUC1-C2	555961-C3	Probe - Hs-SERPINE1-C3
416991	Probe - Hs-IL4R	548451-C2	Probe - Hs-PECAM1 -C2	591721-C3	Probe - Hs-BCL2-C3
419711	Probe - Hs-MYD88	310501-C2	Probe - Hs-IFNG-C2	310311-C3	Probe - Hs-FN1-C3
419741	Probe - Hs-NFE2L2	319391-C2	Probe - Hs-IL5-C2	310391-C3	Probe - Hs-MUC1-C3
416961	Probe - Hs-SDC1	310371-C2	Probe - Hs-IL6-C2	311401-C3	Probe - Hs-CDKN1A-C3
421311	Probe - Hs-SNCA-ver2	555091-C2	Probe - Hs-ITGAM-C2	310421-C3	Probe - Hs-TNFA-C3
420101	Probe - Hs-SPP1	560461-C2	Probe - Hs-VWF-C2	425001-C3	Probe - Hs-PLAU-C3
422831	Probe - Hs-SYK	310421-C2	Probe - Hs-TNFA-C2	548451-C3	Probe - Hs-PECAM1-C3
420771	Probe - Hs-TNC	310061-C2	Probe - Hs-EGFR-C2	311271-C4	Probe - Hs-CD44-C4
417651	Probe - Hs-TNFRSF1B	310311-C2	Probe - Hs-FN1-C2	601501-C4	Probe - Hs-CCR5-C4
423161	Probe - Hs-VEGFA	310761-C2	Probe - Hs-HGF-C2	560461-C4	Probe - Hs-VWF-C4
424321	Probe - Hs-BDKRB1	310931-C2	Probe - Hs-IL17A-C2	310421-C4	Probe - Hs-TNFA-C4
424331	Probe - Hs-BDKRB2	420971-C2	Probe - Hs-BDNF-C2		
425251	Probe - Hs-CXCL2	417631-C2	Probe - Hs-CASP1-C2		

Ordering Information

RNAscope® assays are available in both manual and automated formats, to suit individual lab demand. The manual assay workflow is similar to IHC and only requires a hybridization oven (HybEZ™ System) to help control the temperature and humidity during hybridization. RNAscope® automated assays are available for Ventana® Discovery® XT and Discovery Ultra systems and for Leica Biosystem's BOND RX System.

RNAscope® Reagent Kits

CAT No.	Product Name
310035	RNAscope® 2.0 HD Reagent Kit–BROWN
310036	RNAscope® 2.0 HD Reagent Kit–RED
320700	RNAscope® 2-plex Reagent Kit
320850	RNAscope® Multiplex Fluorescent Reagent Kit
321100	RNAscope® LS Reagent Kit–BROWN for Leica System
320600	RNAscope® VS Reagent Kit–BROWN for Ventana System
320610	RNAscope® VS Reagent Kit–RED for Ventana System

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1. Meng, X., Zhang, Y., Lao, L., Saito, R., Li, A., & Bäckman, C. *et al*. (2013). Spinal interleukin-17 promotes thermal hyperalgesia and NMDA NR1 phosphorylation in an inflammatory pain rat model. *PAIN*, 154(2), 294-305.
2. Østvik, A., vB Granlund, A., Gustafsson, B., Torp, S., Espevik, T., & Mollnes, T. *et al*. (2014). Mucosal Toll-like Receptor 3-dependent Synthesis of Complement Factor B and Systemic Complement Activation in Inflammatory Bowel Disease. *Inflammatory Bowel Diseases,* 1.
3. Brenna, Ø., Furnes, M., Drozdov, I., van Beelen Granlund, A., Flatberg, A., & Sandvik, A. *et al*. (2013). Relevance of TNBS-Colitis in Rats: A Methodological Study with Endoscopic, Histologic and Transcriptomic Characterization and Correlation to IBD. *Plos ONE*, 8(1), e54543.

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