

**CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET**

<b>Product name</b>	RNA:DNA Hybrids	<b>Expiry date</b>	-
<b>Catalog number</b>	HC4072-02		
<b>Lot number</b>	-	<b>Activity</b>	N.A.
<b>Volume</b>	Reconstitute with nuclease-free water, volume depending on the concentration to be used	<b>Amount</b>	25 nmol
<b>Formulation</b>	Lyophilized	<b>Concentration</b>	N.A.
<b>Host Species</b>	Purified 60-mer RNA:DNA hybrids	<b>Purification</b>	N.A.
<b>Endotoxin</b>	<24 EU/mg	<b>Purity</b>	N.A.
<b>Storage</b>	4°C		

**Application notes**

	IHC-F	IHC-P	IF	FC	FS	IA	IP	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; W = Western blot

- RNA:DNA hybrids can be used for cellular stimulation. Furthermore, RNA:DNA hybrids can be used to induce a pro-inflammatory cytokine response in dendritic cells.

**General Information**

**Description** RNA:DNA hybrid represents a novel class of high-affinity ligand for Toll Like Receptor-9 (TLR9). TLRs are expressed by various cells of the immune system, such as macrophages and dendritic cells. TLRs are class I receptors, with a single  $\alpha$ -helix that spans the cell membrane. They recognize and respond to molecules derived from bacterial, viral and fungal pathogens, such as lipopolysaccharide (LPS) from the outer membrane of Gram negative bacteria, peptidoglycan fragments from bacterial cell walls and single-stranded and double-stranded RNA from viruses. Nucleic acids are of course fundamental components of all pathogens. Several nucleic acid recognizing Pattern Recognition Receptors (PRR) have been identified including TLR3 (dsRNA), TLR7 (ssRNA), TLR8 (ssRNA), TLR9 (bacterial DNA) and TLR13 (23S rRNA) confined to the endosomal compartments, membrane bound C-type lectin receptors, the cytosolic Nod-like receptors and cytosolic RIG-like receptors. Nucleic acid binding to TLRs recruits the adaptor proteins TRIF or MyD88 to trigger NF- $\kappa$ B and/or IRF signaling pathways, inducing pro-inflammatory cytokines and type I interferons. Current understanding of TLR9 is primarily determined from the use of synthetic oligodeoxynucleotides. Most well-known is the interaction of TLR9 with non-methylated cytosineguanosine (CpG) motifs in DNA. Activation of TLR9 is ascribed to the detection of CpG-motifs within pathogen derived DNA. TLR9 signaling not only initiates innate immune cells, but also acquired responses. RNA:RNA hybrids have been described as a new ligand for TLR9. Many pathogenic viruses replicate via a RNA:DNA hybrid intermediate within the cell. Viral genomes are detected as non-self and induce a powerful antiviral response via type I interferon. Indeed, RNA:DNA hybrids are sensed by dendritic cells via TLR9. It was demonstrated that TLR9 mediated cytokine response elicited by the hybrid was MyD88 dependent. The ability to modulate TLR9 activity and subsequent innate responses, through DNA-based immunotherapeutics has been the focus of many research projects. Nucleic acids are attractive as therapeutics for their chemical stability and low costs. The ability to regulate downstream signaling by varying the sequences of the synthetic nucleic acid opens an interesting therapeutic window. HC4072 consists of a RNA:DNA hybrid duplex: 60-mer RNA(GU) annealed to 60-mer DNA (CA).

- References**
1. Kailasan Vanaja, S et al; Bacterial RNA:DNA hybrids are activators of the NLRP3 inflammasome. PNAS 2014, 111:7765
  2. Mankan, A et al; Cytosolic RNA:DNA hybrids activate the cGAS–STING axis. The EMBO J 2014, 33:2937
  3. Rigby, A et al; RNA:DNA hybrids are a novel molecular pattern sensed by TLR9. The EMBO Journal 2014, 33:6
  4. Mackenzie, K et al; Ribonuclease H2 mutations induce a cGAS/STINGdependent innate immune response. The EMBO J 2016

**Storage&stability** Caution: vial is under vacuum. Lyophilized product should be stored at 4°C. Store stock solution in aliquots at –20°C. Repeated freeze and thaw cycles will cause loss of activity. 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.

---

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  
30/03/2018

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