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Datasheet for 200-301-MV0

ModDetect® Phosphorothioate Clone PS04

Overview

Description:	ModDetect® Phosphorothioate Clone PS04 - 200-301-MV0
Item No.:	200-301-MV0
Size:	100 µg
Applications:	ELISA, IF, Multiplex
Host Species:	Mouse

Product Details

Background:	Current studies focusing on RNA therapeutics use as potential drugs antisense oligonucleotides (ASO), short interfering RNA (siRNA), and micro RNA (miRNA). The choice of oligonucleotide chosen for a specific treatment is dependent on many factors. However, all oligo therapeutics must be modified in some manner, as unmodified nucleotides are difficult to identify and are highly susceptible to degradation by endogenous nucleases. Modifications can be created within the sugar-phosphate backbone (first generation), the ribose sugar (second and third generation), or at multiple locations (third generation). Antibodies to modified oligonucleotides are useful for basic immunoassays including, ELISA, IHC, and IF. In addition, several types of toxicology assays (immunogenicity assays), such as antibody-drug assays (ADA) or other pharmacokinetic (PK) and pharmacodynamic (PD) studies require oligonucleotide antibodies as analytical tools for general research and preclinical trial experiments. Antibodies to an oligonucleotide therapeutic allow researchers to assess cellular uptake, tissue distribution, and can serve as a positive control for immunogenicity studies.
Synonyms:	Phosphorothioate, PS, ASO, anti-sense oligonucleotide, siRNA, RNA therapeutic
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	PS04
Format:	IgG2a

Target Details

Immunogen Type:	Other

Immunogen:	ModDetect® Phosphorothioate Clone PS04 was prepared from cell culture supernatant produced by repeated immunizations with a proprietary oligo sequence.
Purity/Specificity:	This Protein A purified ModDetect® Phosphorothioate Clone PS04 is directed against phosphorothioate modified backbone. This product was Protein A purified from monospecific supernatant.
Relevant Links:	• 200-301-MV0 SDS • 200-301-MV0 EU SDS

Application Details

Tested Applications:	ELISA, IF, Multiplex
Application Note:	ModDetect® Phosphorothioate Clone PS04 has been validated for use in ELISA. In antigen-down or immunometric ELISAs, the ModDetect® Phosphorothioate Clone PS04 preferentially detects phosphorothioate modified phosphate groups over native phosphodiester sugar-phosphate backbones. It is also likely to be functional in IF and IHC assays, however, its sensitivity, specificity, and conditions for optimal use in these assays has not yet been determined. All assays should be optimized by the user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.

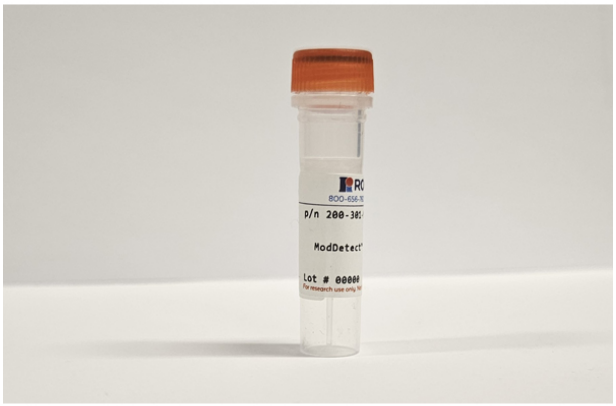
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.00 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free

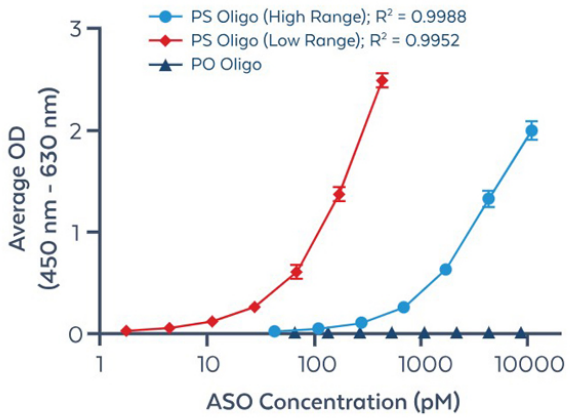
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

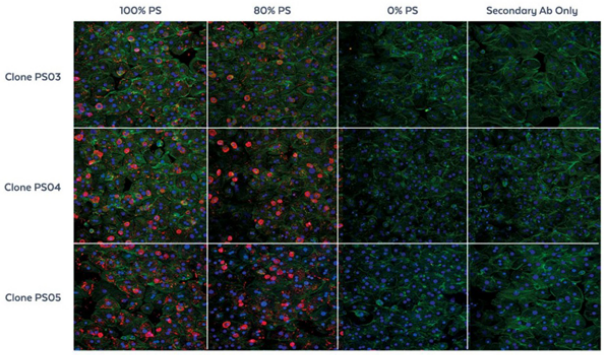
Images



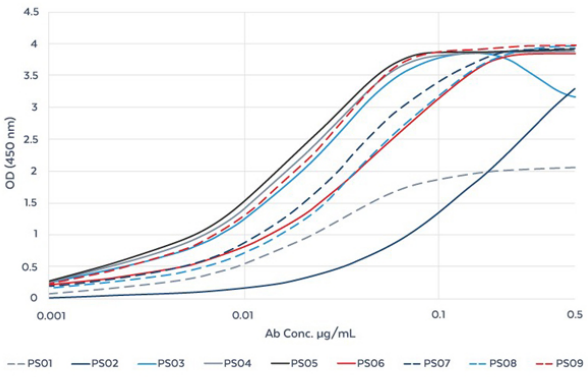
Vial
ModDetect® Phosphorothioate Clone PS04



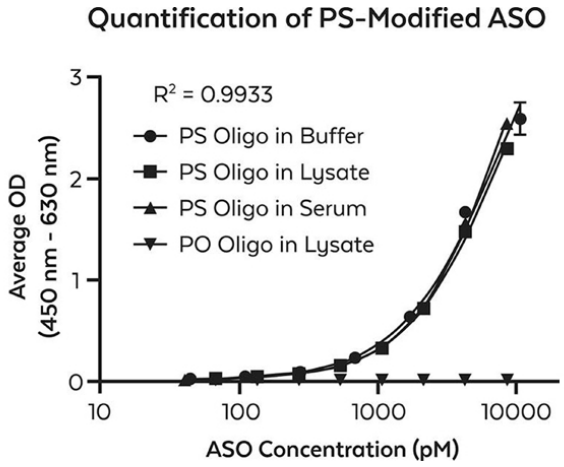
ELISA
Quantification of fully PS modified ASO by ELISA using anti-PS monoclonal antibody.
Both low and high dynamic range curves were generated using PS-modified ASO diluted in buffer and subsequently detected using ModDetect® anti-PS antibody clone PS04 (p/n 200-301-MV0) from 10,000 pM (0.4-100 ng/mL) in concentrations for the low and high range assays, respectively. Non-modified PO oligonucleotide of the same sequence was used as a negative control (PO Oligo). A standard curve was plotted as the average OD result versus the log of ASO concentration in pM using a 4PL best fit formula. The LLOQ as defined as the lowest standard detected is 1.7 pM/44 pM (low/high range). The LOD was determined to be 0.9 pM/<11pM (low/high) based on standard definitions of the term. Fig 4. PMID: 39993214.



Immunofluorescence Microscopy
Biodistribution of ONT drug using selected Anti-PS monoclonal antibodies.
Human hepatocytes were treated with ASO by free uptake and then stained by immunofluorescence microscopy with DAPI (blue), phalloidin (green), and anti-PS antibody (red). ModDetect® Anti-PS antibodies were used at a dilution range of 1:200 to 1:1000. The top panel of images represents staining using clone PS03 (p/n 200-301-MU9), the middle panel clone PS04 (p/n 200-301-MV0), and the bottom panel clone PS05 (p/n 200-301-MV1), respectively. Tissue was treated either with 100% modified ASO, 80% modified ASO, unmodified ASO, or with secondary antibody only as a negative control. Panels for 100% and 80% modified drug show clear staining of cells containing the ASO. The larger cells stained red are believed to be apoptotic hepatocytes.

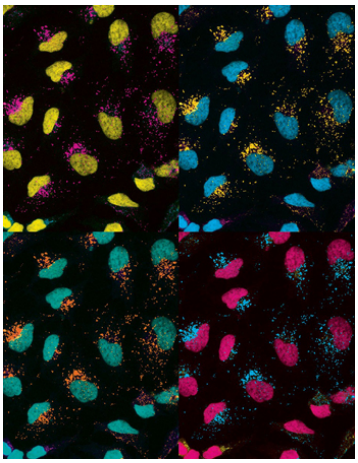


ELISA
ELISA profiles of 9 ModDetect® Phosphorothioate Detection reagents reactive to control oligonucleotides. Fully PS-modified positive control ASOs were used to show differential sensitivity and specificity of ModDetect® Phosphorothioate clones selected for validation. Clones were not reactive with the same sequence synthesized with PO backbones (data not shown). Each well was coated in duplicate with 5 µg/mL of BSA-oligo conjugate. The starting dilution of antibody was 5 µg/mL and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using 3% fish gel, Mouse IgG (gamma 1, 2a, 2b and 3 chain) Antibody Peroxidase Conjugated (p/n 610-403-C46) and TMB ELISA Peroxidase Substrate (p/n TMBE-1000).

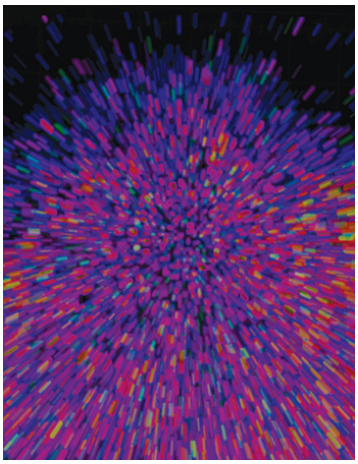


ELISA

Quantification of fully PS-modified ASO by ELISA using anti-PS monoclonal antibody (clone PS04). The ability to quantify PS-modified ASO diluted in tris-based buffer (●) over a range from 44 pM to 11 nM concentrations is shown in the curve as indicated by spiking known amounts of the ASO from a 100 nM stock by 2.5-fold serial dilution and subsequently detected using anti-PS monoclonal antibody. Samples were run in triplicate. A standard curve was plotted as the average OD result vs. the log of ASO concentration in pM using a 4PL best-fit formula. The effect of matrices was investigated by performing similar experiments replacing the tris-based buffer with either HeLa whole cell lysate (□) or mouse serum (□) at initial protein concentrations of 0.3 µg/mL and 2.5 mg/mL, respectively. Nonmodified PO oligonucleotide of the same sequence was used as a negative control (PO Oligo) and was similarly prepared in HeLa whole cell lysate (□). Reproducibility was confirmed by repeating the assay twice (data not shown). For PS-modified ASO diluted in tris-based buffer, the LLOQ defined as the lowest standard detected is 44 pM. The LOD, defined as the average signal of background + 3× standard deviation) was determined to be <33pM. The ULOQ as shown is 11 nM. The LLOQ/ULOQ is defined as the lowest/highest standard concentration meeting the acceptance criteria of calculated/theoretical × 100% recovery between 70% and 130% and CV ≤25% for LLOQ and between 80% and 120% and CV ≤20% for ULOQ. Further optimization and sensitivity enhancement will likely decrease the LLOQ by one log unit. ELISA, enzyme-linked immunosorbent assay; LLOQ, lower limit of quantitation; LOD, limit of detection; ULOQ upper limit of quantitation. Fig. 4. PMID: 39993214



Immunofluorescence Microscopy
Immunofluorescence using ModDetect®. Immunoassays, enabled by highly specific monoclonal antibodies to chemical modifications common to many oligonucleotide therapeutics, empower researchers to consider novel approaches to assess the safety and efficacy of this class of drugs. This image, featured on the cover of OTS Nucleic Acid Therapeutics, Vol. 35, No. 1, February 2025, shows immunofluorescent staining of HeLa cells after gymnotic uptake of a 16-mer LNA gapmer ASO followed by incubation with a monoclonal antibody specific for phosphorothioate modified inter nucleotide linkages. The punctate staining represents the localization of drug within cells. Nuclei are also counterstained. Image provided by Ineˆs Fial, Nucleic Acid Therapy Accelerator (NATA). The caption and Warholian cover were provided by corresponding author, Carl Ascoli of Rockland Immunochemicals, Inc., Limerick, PA, on behalf of the co-authors of the Issues in Development paper, 'Bioanalytical Assays for Oligonucleotide Therapeutics: Adding Antibody-based Immunoassays to the Toolbox as an Orthogonal Approach to LC-MS/MS and Ligand Binding Assays' (Chimento et al., PMID: 39993214).



Immunocytochemistry
Immunocytochemistry using ModDetect®. This image, featured on the cover of OTS Nucleic Acid Therapeutics, Vol. 35, No. 4, August 2025, features a three-dimensional reconstruction with pseudo-color rendering of a spheroid culture with staining for cell nuclei and immunocytochemistry using antibodies to detect endosomes and the phosphorothioate (PS) modification of an antisense oligonucleotide. These cultures were used as part of a benchmarking study in cells and tissue to assess the ModDetect® series of antibodies raised against PS and 2'-MOE.
Image provided by Peter Oliver of the Medical Research Council Nucleic Acid Therapy Accelerator, Research Complex at Harwell, Didcot, UK.

References

- Chimento DP et al. Bioanalytical Assays for Oligonucleotide Therapeutics: Adding Antibody-Based Immunoassays to the Toolbox as an Orthogonal Approach to LC-MS/MS and Ligand Binding Assays. *Nucleic Acid Ther.* (2025)
- Fial I et al. Characterizing Antibodies Targeting Antisense Oligonucleotide Phosphorothioate and 2'- O-Methoxyethyl Modifications for Intracellular Trafficking and Biodistribution Studies. *Nucleic Acid Ther.* (2025)

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