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Datasheet for KNA-100

ModDetect® Phosphorothioate Panel

Overview

Description:	ModDetect® Phosphorothioate Panel - KNA-100
Item No.:	KNA-100
Size:	1 Set
Applications:	ELISA, IF, Multiplex

Product Details

Synonyms:	Phosphorothioate, PS, ASO, anti-sense oligonucleotide
Detection Kit Type:	Combo Pack

Application Details

Tested Applications:	ELISA, IF, Multiplex
Application Note:	The set includes five unlabeled ModDetect® phosphorothioate detection reagents [clone PS03 (200-301-MU9S), clone PS04 (200-301-MV0S), clone PS05 (200-301-MV1S), clone PS07 (200-301-MW0S), and clone PS09 (200-301-MW3S)] each supplied as a 25 µL aliquot at approximately 1.0 mg/mL, curated by binding characteristics, and three rabbit Anti-Mouse IgG reporter molecules labeled with peroxidase (610-403-C46S), 488 λ fluorophore (610-441-C46), and 649 λ fluorophore (610-443-C46) each supplied as 100µg. The different binding characteristics create opportunities to generate highly optimized immunoassays independent of oligonucleotide sequence, as opposed to spending significant efforts to optimize assays to underperforming reagents. Designed for use in various immunoassays, such as ELISA, IF, and IHC. NOTE: Panel detection reagents components are provided in a 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride buffer, pH 7.2. This buffer contains 0.01 % Sodium Azide as a preservative and 10 mg/mL carrier protein as a stabilizing reagent.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	User Optimized
IF:	User Optimized

IHC:	User Optimized

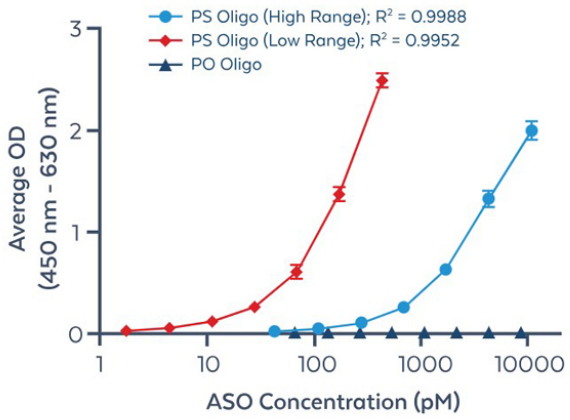
Formulation

Physical State:	Liquid and Lyophilized
Concentration:	None

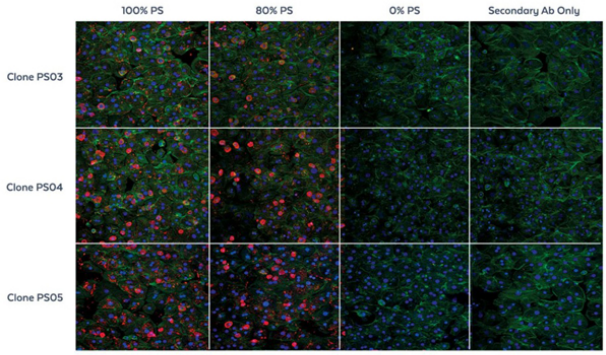
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Please see product page for complete product description. Store five phosphorothioate vials at -20°C or below prior to opening. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing. Store three reporter molecule vials at 4°C or -20°C prior to restoration. For extended storage aliquot contents and freeze at -20°C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4°C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

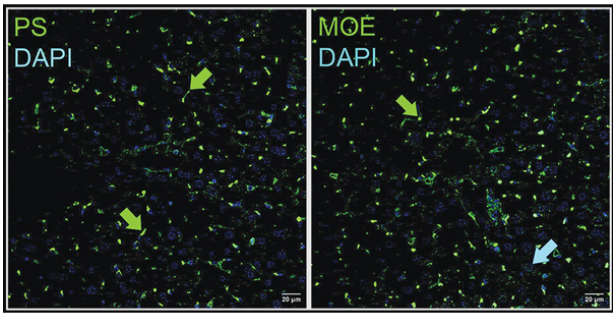
Images



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.

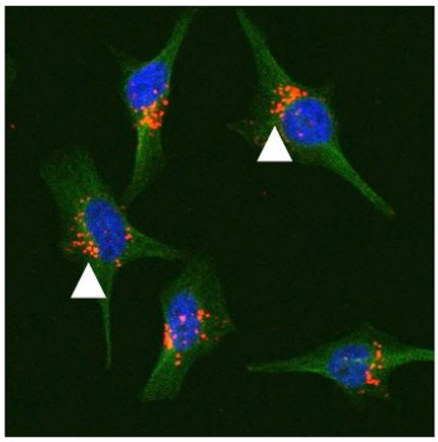


Immunofluorescence Microscopy
Biodistribution of ONT drug using selected Anti-PS monoclonal antibodies.
Human hepatocytes were treated with ASO by free uptake and then stained by immunofluorescence microscopy with DAPI (blue), phalloidin (green), and anti-PS antibody (red). ModDetect® Anti-PS antibodies 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.

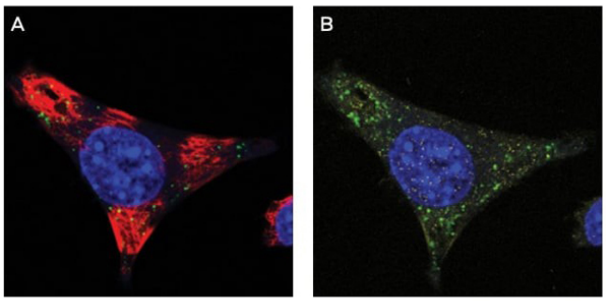


Immunofluorescence Microscopy
Immunohistochemistry of a systemically delivered ASO in mice.
A 2'-MOE modified gapmer with PS bonds (MOE/PS) was delivered subcutaneously to mice and fixed liver tissue immunostained with ModDetect® anti-PS clone PS03 (p/n 200-301-MU9) or anti-MOE clone MOE4 (p/n 200-301-NF2) antibodies independently as indicated. Representative positive immunostaining (green arrows) indicates accumulation of the ASO in nonparenchymal cells surrounding hepatocytes (blue arrows indicate example large hepatic nuclei, DAPI). Images taken by confocal microscopy at 20x. Scale bars as indicated. FIG. 5A.
PMID: 40685980.

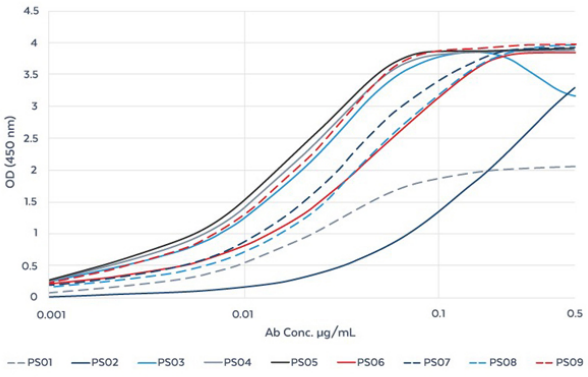
Kit Box
This ModDetect® Phosphorothioate Panel includes the following components: five unlabeled ModDetect® phosphorothioate detection reagents, PS03, PS04, PS05, PS07, PS09, and three reporter molecules labeled with peroxidase, 488 λ fluorophore and 649 λ fluorophore.



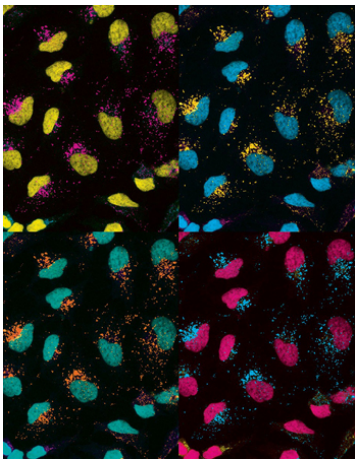
Immunofluorescence Microscopy
ModDetect® PS03 (p/n 200-301-MU9) detects ASO59. (A) IF of HeLa cells following free uptake (1 µM) of ASO59 for 24 h. Arrowheads highlight the ModDetect® PS03 signal. Images are at 20ξ magnification with zoomed-in frames. PS03 (red), Tubulin (green), DAPI (blue). Citation Krainer A. Figure S5.



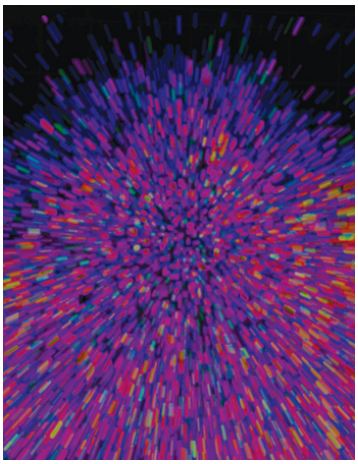
Immunofluorescence Microscopy
Immunofluorescence of ModDetect® Reagent PS05. Mouse glioma cells derived from C57 black mice were cultured and treated with Oligo Tx drug. After fixation with paraformaldehyde, cells were stained with alpha tubulin (red) and ModDetect® reagent PS05 (green). Reagent PS05 was used at a 1:2000 dilution. Punctate cytoplasmic staining is consistent with endosomal storage of ASO within the cell, as expected for this Oligo Tx drug. Vehicle only treated cells showed no staining (not shown).



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).



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)
- Kashyap D et al. Harnessing BET-Bromodomain Assisted Nuclear Import for Targeted Subcellular Localization and Enhanced Efficacy of Antisense Oligonucleotides. *J Am Chem Soc.* (2025)

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