Using Morpholinos & Vivo-Morpholinos

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MSDS are posted at: www.gene-tools.com/MSDS

USE

Oligo state on shipment

Standard Morpholino oligos and Vivo-Morpholinos are shipped as sterile lyophilized solids.

Making a Stock Solution of a Morpholino

A Morpholino is delivered as a prequantitated, sterile, salt-free, lyophilized solid in a glass vial. We recommend making a ≤1 mM Morpholino stock solution in distilled water. Like other oligos, Morpholinos can be damaged by diethyl pvrocarbonate (DEPC).

Vivo-Morpholinos, due to their lower solubilities, should be dissolved at lower concentration (≤0.5 mM). Water can be removed by lyophilization if needed. It is easiest to analyze Morpholinos by MALDI-TOF from a water solution. You can make a stock solution in a buffer (e.g. Danieau buffer or Ringer's solution); however, some salts decrease solubility of Vivo-Morpholinos and lyophilization or mass spectrometry will be more difficult. Delivery dyes might inhibit Vivo-Morpholino activity. Do not autoclave Vivo-Morpholino solutions more than once.

GENE TOOLS suggests storing Morpholino stock solutions at room temperature in the original GENE TOOLS vial. It is best to keep stocks at 1 mM or less to avoid insolubility or aggregation and in tightly sealed vials to prevent evaporation, ideally in a humid environment. Activity lost due to solubility issues can often be restored by autoclaving Morpholino solutions. If your oligo does not readily dissolve during stock preparation, heat the vial to 65°C and vortex. If dissolution is incomplete, autoclave the stock solution.

Stock solutions of Morpholino oligos			
Amount of Morpholino	Volume of sterile		
(Amount of Vivo-Morpholino)	water	concentration	
100 nanomoles	0.10 mL	1.0 milliMolar (mM)	
(100 nanomoles Vivo-MO)	0.20 mL	0.5 milliMolar (mM)	
300 nanomoles	0.30 mL	1.0 milliMolar (mM)	
(400 nanomoles Vivo-MO)	0.80 mL	0.5 milliMolar (mM)	
1000 nanomoles	1.00 mL	1.0 milliMolar (mM)	
(2000 nanomoles Vivo-MO)	4.00 mL	0.5 milliMolar (mM)	

Oligo Concentrations

Typical effective concentrations of standard Morpholino oligos in various systems:

Test system	Oligo concentration	
Electroporation in cultures	1 μM to 10 μM (in delivery solution)	
Endo-Porter ^(a) in cultures	1 μM to 10 μM (in medium)	
Scrape-loading ^(b) in cultures	1 μM to 20 μM (in medium)	
Microinjection into oocytes	Inject 1 to 10 nanoliters of 1 mM oligo into 1 μl oocyte to give 1 to10 μM final concentration in oocyte	
Cell-free translation system ^(c)	100 nM to 1000 nM (in lysate)	

- (a) Endo-Porter solution delivers Morpholino oligos into the cytosol of cells efficiently and uniformly by releasing oligos from endosomes.
- (b) Morpholino oligos may be loaded into the cytosol/nuclear compartment of adherent cells by adding oligo to the medium and then scraping the cells from the plate (see: Antisense and Nucleic Acid Drug Dev. **6**, 166 (1996)).
- (c) See: Antisense and Nucleic Acid Drug Dev. 7, 63 (1997)

Typical effective concentrations of Vivo-Morpholino oligos in various systems:

Test system	Oligo concentration and/or dose
Cell bathing in cultures	1-10 µM in nutrient medium
Injection into mouse tail vein	12.5 (mg oligo)/(kg mouse) per day
Injection into mouse intraperitoneal	12.5 (mg oligo)/(kg mouse) per day

Cell Delivery Protocols

Upon request GENE TOOLS will provide protocols for Endo-Porter or scrape delivery with cell scrapers. Copies of these protocols are normally shipped with orders which include these products.

QUANTITY

Standard Morpholino oligos

Typical package size for a classic Morpholino oligo is:

300 nanomoles (about 2.5 mg or 75 OD units for 25-mer).

Larger amounts available (1000 nanomole, 6000 nanomole, 1g, etc.).

Vivo-Morpholino oligos

Typical package size for a Vivo-Morpholino oligo is:

400 nanomoles (about 4 mg or 100 OD units for 25-mer).

Larger amounts available (2000 nanomole, 10000 nanomole, 1g, etc.).

Note: The quantities above are the <u>measured and delivered amounts</u> of lyophilized, sterile Morpholino oligos.

GENE TOOLS PREPARED CONTROLS

Standard Control

Sequence:

5' CCTCTTACCTCAGTTACAATTTATA 3'

Calculated Mass: 8328 Molar Absorbance

@265 nm in 0.1 N HCI: 259,160 1/(M*cm)

Quantity Delivered: 100 nanomoles

25.9 OD 265, pH1 Units

0.833 mg

Fluoresceinated Standard Control Sequence:

5' CCTCTTACCTCAGTTACAATTTATA 3'

Calculated Mass: 8817 Molar Absorbance

@265 nm in 0.1 N HCI: 259,160 1/(M*cm) **Quantity Delivered**:100 nanomoles

25.9 OD _{265, pH1} Units

0.882 mg

Lissaminated Standard Control

Sequence:

5' CCTCTTACCTCAgTTACAATTTATA 3'

Calculated Mass: 9112 Molar Absorbance

@265 nm in 0.1 N HCI: 259,160 1/(M*cm)

Quantity Delivered:100 nanomoles

 $25.9 \; OD \; {}_{265,\; pH1} \; Units$

0.911 mg

Vivo Standard Control

Sequence:

5' CCTCTTACCTCAGTTACAATTTATA 3'

Calculated Mass: 10,138

Molar Absorbance

@265 nm in 0.1 N HCI: 259,160 1/(M/cm) Quantity Delivered:100 nanomoles

25.9 OD _{265, pH1} Units

1.01 mg

Gene Tools Blue Standard Control Sequence:

5' CCTCTTACCTCAGTTACAATTTATA 3'

Calculated Mass: 9078 Molar Absorbance

@265 nm in 0.1 N HCI: 259,160 1/(M*cm)

Quantity Delivered:100 nanomoles

25.9 OD _{265, pH1} Units

0.908 mg

Random Control 25-N

Sequence:

Calculated Mass: 8463 average mix of 25-mer

Molar Absorbance

@265 nm in 0.1 N HCI: 259,063 1/(M*cm)_{avg}

Quantity Delivered:100 nanomoles

25.9 OD _{265, pH1} Units

0.846 mg (average)

Zebrafish p53

Sequence:

5' GCGCCATTGCTTTGCAAGAATTG 3'

Calculated Mass: 7805 Molar Absorbance

@265 nm in 0.1 N HCI: 236,990 1/(M*cm)

Quantity Delivered:100 nanomoles

23.7 OD 265, pH1 Units

0.780 mg

Zebrafish Chordin (3'Fluorescein)

Sequence:

5' ATCCACAGCAGCCCCTCCATCATCC 3'

Calculated Mass: 8742 Molar Absorbance

@265 nm in 0.1 N HCI: 250,880 1/(M*cm)

Quantity Delivered: 100 nanomoles

25.1 OD _{265. pH1} Units

0.876 mg

Clawed Frog p53

Sequence:

5' GCCGGTCTCAGAGGAAGGTTCCATT 3'

Calculated Mass: 8500 Molar Absorbance

@265 nm in 0.1 N HCI: 256,200 1/(M*cm)

Quantity Delivered: 100 nanomoles

25.6 OD _{265, pH1} Units

0.850 mg

Clawed Frog Beta-Catenin (3'Fluorescein) Sequence:

5' TTTCAACCGTTTCCAAAGAACCAGG 3'

Calculated Mass: 8901 **Molar Absorbance**

@265 nm in 0.1 N HCI: 262,740 1/(M*cm) Quantity Delivered:100 nanomoles

24.3 OD 265, pH1 Units

0.890 mg

Green Flourescent Protein Positive Ctrl Sequence:

5' ACAGCTCCTCGCCCTTGCTCACCAT 3'

Calculated Mass: 8275 **Molar Absorbance**

@265 nm in 0.1 N HCI: 246,420 1/(M*cm)

Quantity Delivered:100 nanomoles

24.6 OD 265, pH1 Units

0.828 mg

Vivo GFP Positive Control

Sequence:

5' ACAGCTCCTCGCCCTTGCTCACCAT 3'

Calculated Mass: 10.085

Molar Absorbance

@265 nm in 0.1 N HCI: 246,420 1/(M*cm) **Quantity Delivered**:100 nanomoles

24.6 OD _{265, pH1} Units

1.01 mg

Photo GPF Postive Control

Sequence:

5` ACAGCTCCTCaaPaaTGCTCACCAT 3`

Calculated Mass: 8237 **Molar Absorbance**

@265 nm in 0.1 N HCI: 255,910 1/(M*cm) **Quantity Delivered**:100 nanomoles

25.6 OD 265, pH1 Units

0.824 mg

Gal4-UAS Photo Morpholino Antisense Sequence:

5` GTTCGATAGAtaPatGTAGCTTCAT 3`

Calculated Mass: 8393 **Molar Absorbance**

@265 nm in 0.1 N HCI: 259,160 1/(M*cm) Quantity Delivered:100 nanomoles

25.9 OD _{265, pH1} Units

0.839 ma

Gal4-UAS Photo Morpholino Sense

Sequence:

5' ATGAAGCTACaaPaaTCTATCGAAC 3'

Calculated Mass: 8349 **Molar Absorbance**

@265 nm in 0.1 N HCI: 268,450 1/(M*cm) **Quantity Delivered**:100 nanomoles

25.95 OD 265, pH1 Units

0.835 mg

Gal4-UAS

Sequence:

5' GTTCGATAGAAGACAGTAGCTTCAT 3'

Calculated Mass: 8507 **Molar Absorbance**

@265 nm in 0.1 N HCI: 266,090 1/(M*cm) **Quantity Delivered**:100 nanomoles 26.6 OD 265, pH1 Units

0.851 mg

Material Safety Data Sheet

Morpholino Phosphorodiamidate Oligomer

Revision Date: Apr 2002

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Section I: Product Identification

Product Name: Morpholino Oligos

Chemical Name: Morpholino Phosphorodiamidate Oligomer

Chemical Formula: N/A CASS #: None assigned

Section II: Hazardous Ingredients

Hazardous Components: None

Section III: Physical/Chemical Characteristics

Physical State: White Lyophilized Powder

Odor: None

Boiling Point: Does not boil Vapor Pressure: Negligible Vapor Density: N/A Solubility: Soluble in water Specific Gravity: Not determined Melting Point: Does not melt Evaporation Rate: Negligible

Section IV: Fire and Explosion Hazard Data

Flash Point: No information available Unusual Fire or Explosion Hazards: None

Extinguishing Media: Fire-Fighting Instructions: Use water

Section V: Reactivity Data

Chemical Stability: Stable under normal temperatures and pressures Incompatibility: Strong acids cause non-hazardous degradation of product

Conditions to Avoid: None reported Hazardous Polymerization: Will not occur

Section VI: Health Hazard Data

Routes of Entry: May enter the body through ingestion, inhalation, skin and eye contact

Carcinogenicity: No information available

Toxicity: Intravenous injection of up to 800 mg/kg in mice causes no acute toxicity Health Hazards: Preliminary studies suggest this product is not a health hazard

Signs and Symptoms of Exposure: Unknown, handle with care

Emergency and First Aid Procedures: None required

Section VII: Precautions for Safe Handling and Storage

Storage Precautions: Store at or below room temperature

Steps to be taken in case material is released or spilled: Wash area with soap and water Waste Disposal Method: Observe all Federal, State and Local Environmental Regulations

Section VIII: Control Measures

Respiratory Protection: None required Ventilation: General ventilation is sufficient Personal Protective Equipment: None required Work/Hygiene Practices: None required

The above information is correct to the best of our knowledge. This material is intended for research purposes only and must only be used under the supervision of a person experienced in handling hazardous materials. Gene Tools, LLC makes no guarantee of the accuracy or completeness of the information and shall not be held liable for any damage resulting from handling or from contact wit the above material.

Morpholino Oligos are covered by European and United States Patents, including: 5,142,047 and 5,185,444.

Material Safety Data Sheet

Vivo-Morpholino Oligomer Date Updated: 10 June 2016 GENE TOOLS, LLC

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Section I: Product Identification

Product Name: Vivo-Morpholino Oligo

Chemical Name: Morpholino Phosphorodiamidate Oligomer Conjugated with in vivo Delivery Moiety

Chemical Formula: N/A CAS #: None assigned

Section II: Hazardous Ingredients

Emergency Overview

Caution The chemical, physical, and toxicological

properties of this product have not been thoroughly investigated. Exercise due care.

HMIS Rating

Health 3
Flammability 0
Reactivity 0
NFPA Rating
Health 3
Flammability 0
Reactivity 0

Section III: First Aid Measures

Oral Exposure If swallowed, wash out mouth with water provided

person is conscious. Call a physician. If inhaled, remove to fresh air. If breathing

Inhalation Exposure If inhaled, remove to fresh air. If brea becomes difficult, call a physician.

Dermal Exposure In case of skin contact, flush with copious

amounts of water for at least 15 minutes. Remove

contaminated clothing and shoes. Call a

physician.

Eye Exposure In case of contact with eyes, flush with copious

amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with

fingers. Call a physician.

Section IV: Fire Fighting Measures

Flash Point Not available
Autoignition Temp Not available
Flammability Not available

Extinguishing Media

Suitable Carbon dioxide, dry chemical powder, or

appropriate foam. Water spray

Fire Fighting

Protective Equipment Wear self-contained breathing apparatus and

protective clothing to prevent contact with skin

and eyes.

Specific Hazard(s) Emits toxic fumes under fire conditions.

Section V: Accidental Release Measures

Procedure to be Followed in Case of Leak or

Spill

Evacuate area

Procedure(s) of Personal Precaution(s) Wear respirator, chemical safety goggles, rubber

boots, and heavy rubber gloves

Methods for Cleaning up Sweep up, place in a bag and hold for waste

disposal. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

Section VI: Handling and Storage

Handling

User Exposure Avoid inhalation. Avoid contact with eyes,

skin, and clothing. Avoid prolonged or

repeated exposure.

Storage

Suitable Keep tightly closed

Storage at or below room temperature

Section VII: Exposure Controls/Personal Protection Equipment

Engineering Controls Mechanical exhaust required

Personal Protective equipment

Respiratory Protection is not required. Where

protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143)

respirator.

Other Wear appropriate government approved

respirator, chemical-resistant gloves, safety

goggles, other protective clothing.

General Hygiene Measures Wash thoroughly after handling

Section VIII: Physical/Chemical Characteristics

Physical State: Lyophilized solid

Odor: None

Boiling Point:

Vapor Pressure:

Vapor Density:

Not determined

Not determined

N/A

Solubility: Soluble in water Specific Gravity: Not determined Evaporation Rate: Negligible

Section IX: Stability and Reactivity

Stability

Stable stable

Hazardous Decomposition Products

Hazardous Decomposition Products Carbon monoxide, carbon dioxide, nitrogen

oxides

Hazardous Polymerization

Hazardous Polymerization Will not occur

Section X: Toxicological Information

Route of Exposure

Skin Contact May cause skin irritation

Skin Absorption May be harmful if absorbed through the skin Inhalation Material may be irritating to mucous membranes

and upper respiratory tract, may be harmful if

inhaled.

Ingestion
Conditions Aggravated by Exposure

May be harmful if swallowed
The toxicological properties have not been

thoroughly investigated.

Section XI: Ecological Information

No data available.

Section XII: Disposal Considerations

Appropriate Method of Disposal of Substance or

Preparation

Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section XIII: Transport Information

DOT

Proper Shipping Name None

Non-Hazardous for Transport This substance is considered to be non-

hazardous for transport.

IATA

Non-Hazardous for Air Transport Non-hazardous for air transport

Section XIV: Regulatory Information

US Classification and Label Text

US Statements Caution: The chemical, physical, and toxicological

No

properties of this product have not been thoroughly investigated. Exercise due care.

United States Regulatory information

SARA Listed

Canada Regulatory information

WHMIS Classification

This product has been classified in accordance with the hazard criteria of the CPR, and the

MSDS contains all the information required by the

CPR.

DSL No NDSL No

Section XV: Other Information

The above information is correct to the best of our knowledge. This material is intended for research purposes only and must only be used under the supervision of a person experienced in handling hazardous materials. GENE TOOLS, LLC makes no guarantee of the accuracy or completeness of the information and shall not be held liable for any damage resulting from handling or from contact with the above material.