SIGNALCHEM DIAGNOSTICS

Catalog #	Aliquot Size
RTH02-E311-2000	2000 U

PureScript II Pro Reverse Transcriptase

Catalog # RTH02-E311
Lot # 1L2208-3

Product Description

PureScript II Pro Reverse Transcriptase is an M-MLV(H-) derived reverse transcriptase expressed in *E. coli*.

Components

	Component Name	2000 U
	PureScript II Pro Reverse Transcriptase (200 U/µl)	10µl
b	5X PureScript II Buffer	500µl

Storage and Stability

Store all components at –30°C to –15°C. To avoid repeated handling and multiple freeze/thaw cycles aliquot product into smaller quantities.

Scientific Background

PureScript II Pro Reverse Transcriptase is a next generation reverse transcriptase derived from the M-MLV (H-) Reverse Transcriptase. Compared with previous generations of reverse transcriptase, the thermo-stability of this product is significantly improved. The half-life of PureScript II Pro Reverse Transcriptase at 50°C is >240 min. At 55°C, the half-life of the enzyme is >1 hour, which significantly benefits the transcription of RNA templates with complex secondary structure. In addition, it has improved template affinity and cDNA synthesis efficiency. It has good resistance to most RT-PCR inhibitors and is suitable for long-fragment cDNA amplification (up to 20 kb). PureScript II Pro Reverse Transcriptase is applicable for reverse transcription of animal, microbial, and plant RNA samples rich in polysaccharides and polyphenols.

Activity

The activity of PureScript II Pro Reverse Transcriptase was determined to be 200 Units/µl, using the unit definition below:

Unit Definition:

One unit (U) is defined as the amount of enzyme that incorporates 1 nmol of dTTP into acid-insoluble material in 10 min at 37°C with Poly (rA)-Oligo (dT) as the template/primer.

This product is manufactured in an ISO 9001 and ISO 13485 certified facility.

PureScript II Pro Reverse Transcriptase

Catalog #	RTH02-E311
Lot #	1L2208-3
Activity	200 Units/µl
Stability	2yrs from date of shipment at –30°C to –15°C
Storage & Shipping	Store all components at -30°C to -15 °C. Transport at ≤0 °C. To avoid repeated handling and multiple freeze/thaw cycles aliquot product into smaller quantities.

To place your order, please contact us by phone 1-778-326-0223 or 1-888-606-3424 (Toll free)
or by email: orders@signalchemdx.com or info@signalchemdx.com - www.signalchemdx.com

cDNA Synthesis Protocol

Notes:

Prevent RNase contamination

Keep the experiment area clean. Wear disposable gloves and masks and use RNase-free tubes and tips.

Primer selection

1. If cDNA products will be used for **PCR**
   - For eukaryotic RNA templates, Oligo dT is generally preferred, which can be paired with 3' Poly A tail of eukaryotic mRNA to obtain the highest yield of full-length cDNA.
   - Use of gene-specific primer (GSP) can obtain the highest specificity. However, switch to Oligo dT or random hexamers if GSP fails in 1st strand cDNA synthesis.
   - Random hexamers have the lowest specificity, and all RNAs, including mRNA, rRNA, and tRNA can be used as templates. Random hexamers can be used as primers when the target region has complex secondary structure or high GC content, or the template is of prokaryotic origin, and Oligo dT or GSP cannot effectively guide cDNA synthesis.
2. If cDNA products will be used for **qPCR**
   - Using a mixture of Oligo dT and random hexamers can make the cDNA synthesis efficiency of each region of mRNA the same, which helps to improve the authenticity and reproducibility of the quantitative results.

cDNA Synthesis for PCR Protocol

1. RNA Template Denaturation*

Prepare the following mixtures in RNase-free tubes.

Component	Amount / Volume
Oligo $(dT)_{23}VN$ (50 µM) or Random hexamers (50 ng/ml) or Gene Specific Primers (2 µM)	1 µl
Total RNA	10pg – 5µg
or Poly (A)+ RNA	10pg – 500ng
RNase-free ddH_2O	to 13 µl

Incubate at 65°C for 5 min and then chill on ice immediately for 2 min.

*RNA template denaturation helps to open secondary structure, which benefits the first-strand cDNA yield. Do not skip the denaturation step if cDNA fragments are longer than 3 kb.

2. Preparation of 1st strand cDNA synthesis reaction mixture

Component	Volume
Mixture from Step 1	13 µl
5X PureScript II Pro Buffer	4 µl
dNTP Mix (10 mM each)	1 µl
PureScript II Pro Reverse Transcriptase (200 U/µl)	1 µl
RNase Inhibitor (40 U/µl)	1 µl

Gently pipette up and down several times to mix thoroughly.

3. Run the following program for 1st strand cDNA synthesis reaction

Temperature	Time
25°C[a]	5 minutes
50°C[b]	45 minutes
85°C	2 minutes

[a.] This step is only necessary when using random hexamers. omit this step when using Oligo $(dT)_{23}VN$ or gene specific primers.
[b.] For templates with complex secondary structure or high GC content, the temperature can be increased to 55°C to improve the yield.

The products can be used for PCR immediately or be stored at -20°C for up to 6 months. It is recommended to store at -70°C after aliquoting for long term storage. Avoid repeated freeze-thaw of cDNA.

cDNA Synthesis for qPCR Protocol

1. Preparation of 1st strand cDNA synthesis reaction mix

Mix the following components in an RNase-free tube.

Component	Volume
5X PureScript II Pro Buffer	4 µl
PureScript II Pro Reverse Transcriptase (200 U/µl)	1 µl
dNTP Mix (10 mM each)	1 µl
RNase Inhibitor (40 U/µl)	1 µl
Oligo $(dT)_{23}VN$ (50 µM)	1 µl
Random hexamers (50 ng/µl)	1 µl
Total RNA	10 pg –-1 µg
or Poly (A)+ RNA	10 pg – 100 ng
RNase-free ddH_2O	to 20µl

Gently pipette up and down several times to mix thoroughly.

2. Run the following program for 1st strand cDNA synthesis

Temperature	Time
25°C	5 minutes
50°C*	15 minutes
85°C	2 minutes

* For templates with complex secondary structure or high GC content, the temperature can be increased to 55°C to improve the yield.

The products can be used for qPCR immediately or stored at -20°C for up to 6 months. However, it is recommended to store at -70°C after aliquoting for long term storage. Avoid repeated freeze-thaw of cDNA.

SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: PureScript II Pro Reverse Transcriptase **Catalog # RTH02-E311**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Supplier of Datasheet:	SignalChem Diagnostics Inc.
Street Address:	*190-13160 Vanier Place*
City, Prov. Postal Code:	*Richmond, BC, V6V 2J2*
Country:	*Canada*
Emergency Phone:	1-888-606-3424 (Toll free) 1-778-326-0223 (local)

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Not GHS classified.
- **Hazard Pictograms:** No labelling applicable.
- **Signal words:** None.
- **Hazard statements:** None.
- **Precautionary statements:** Wear protective gloves/protective clothing/eye protection/ face protection. Avoid breathing dust. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- **Other hazards:** May cause eye and skin irritation. May cause respiratory and digestive tract irritation.

Article 3 – Composition/Information on Ingredients

Description: This product consists of the components listed below.

Component: PureScript II Pro Reverse Transcriptase
Chemical Characterization: Mixture.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	≤50%

Component: 5X PureScript II Buffer
Not a hazardous substance or mixture

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breath in fresh air. If casualty cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Remove contact lenses, if present and easy to do so. Consult a physician.
- **After swallowing:** Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

SAFETY DATA SHEET

Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment, and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store according to product label instructions. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
  Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m3 for mist	British Columbia, Canada
TWA	3 mg/m3 for respirable mist	British Columbia, Canada
TWA	10 mg/m3	Alberta, Canada
TWAEV	10 mg/m3	Ontario, Canada
TWAEV	10 mg/m3	Quebec, Canada
TWA	10 mg/m3	USA

- **Appropriate engineering controls:**
  Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**

Respiratory protection:
Use appropriate respirator if there is inadequate ventilation by following the government standards.

Hand protection:
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.

Eye/face protection:
Safety goggles with side-shields approved under appropriate government standards.

Skin/body protection:
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Component: PureScript II Pro Reverse Transcriptase

Appearance: Colorless fluid.	**Danger of explosion:** Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	**Explosion limits:** Not determined.
pH: ~7.2	**Decomposition temperature:** Not available.
Melting point/freezing point: Not determined.	**Vapor pressure at 20 °C:** Not determined.
Boiling point/Boiling range: 106 °C.	**Density:** ~1.12g/cm^3.
Flash point: Not determined.	**Relative density:** Not determined.
Flammability (solid, gaseous): Not determined.	**Vapor density:** Not determined.
Ignition temperature: Not determined.	**Evaporation rate:** Not determined.
Auto-igniting: Product is not self-igniting.	**Solubility in / Miscibility with Water:** Fully miscible.

Component: 5X PureScript II Buffer

Appearance: Colorless fluid.	**Danger of explosion:** Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	**Explosion limits:** Not determined.
pH: ~8.3	**Decomposition temperature:** Not available.
Melting point/freezing point: Not determined.	**Vapor pressure at 20 °C:** Not determined.
Boiling point/Boiling range: ~100 °C.	**Density:** 1.02g/cm^3.
Flash point: Not determined.	**Relative density:** Not determined.
Flammability (solid, gaseous): Not determined.	**Vapor density:** Not determined.
Ignition temperature: Not determined.	**Evaporation rate:** Not determined.
Auto-igniting: Product is not self-igniting.	**Solubility in / Miscibility with Water:** Fully miscible.

SAFETY DATA SHEET

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Not determined.
- **Hazardous decomposition products:** Not determined.

Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
  Inhalation: No data available
  Ingestion: No data available
  Skin: No data available
  Eyes: No data available
- **Signs and Symptoms of Exposure:** No data available
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** No data available.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 – Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.