Custom TaqMan® Small RNA Assays

DESIGN AND ORDERING GUIDE

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ThermoFisher SCIENTIFIC

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Revision history: Pub. No. 4412550

Revision	Date	Description
F	6 June 2019	Updated for general style, formatting, and branding.
E	January 2011	Baseline for this revision history.

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Contents

1 Product information

Custom TaqMan® Small RNA Assays

Applied Biosystems™ Custom TaqMan® Small RNA Assays are small RNA assays designed to your specifications based on the chemistries for TaqMan® MicroRNA Assays. You can use the custom assays to verify and quantify small RNA sequences, including the following items:

- Mature microRNA (miRNA)
- Small interfering RNA (siRNA)
- Processed short hairpin RNA (shRNA)
- Piwi-interacting RNA (piRNA)
- Any other small RNA sequence that is 17 to 200 bases long

The assays are ordered at **thermofisher.com/taqmancustommirna**.

Description of the assays

The assays use a stem-looped primer for reverse transcription (RT) and a sequence-specific TaqMan® real-time PCR assay to detect small RNAs.

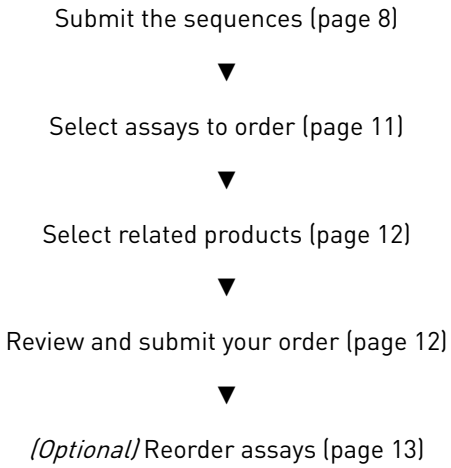
Each assay includes the following components:

- One tube containing a stem-loop RT primer specific to the small RNA of interest
- One tube containing a mixture of the following components:
  - A small RNA-specific forward PCR primer
  - A specific reverse PCR primer
  - A TaqMan® MGB Probe specific to the small RNA of interest

Table 1 Custom TaqMan® Small RNA Assays

Cat. No.	Number of 20–µL reactions	Amount and concentration	
		RT Primer	Assay
4440418 (Extra small)	25 (RT) 75 (PCR)	75 µL (5×)	75 µL (20×)
4398987 (Small)	50 (RT) 150 (PCR)	150 µL (5×)	150 µL (20×)
4398988 (Medium)	750 (RT) 750 PCR	575 µL (20×)	750 µL (20×)
4398989 (Large)	2900 (RT) 2900 (PCR)	725 µL (60×)	967µL (60×)

Workflow



2 Order the assays

Guidelines

Sequence design guidelines

Sequences are entered directly into the design tool or uploaded in a TXT file in FASTA format. The following guidelines must be followed for either method:

- Sequences must contain only A, C, G, T, and U bases.
- Sequences must be between 17 and 200 bases.

  Note: All sequences are designed to the 3' end of the sequence, regardless of the length of the sequence submitted. If your sequence is up to 61 bases, consider ordering a Custom TaqMan® Gene Expression Assay instead of a Custom TaqMan® Small RNA Assay.
- For double-stranded sequences that contain chemically modified bases, submit the sequence of the unmodified strand.

  Note: Chemical modifications of bases can affect the performance of the siRNA assays.
- Limit the name to 20 characters.
- Names cannot include spaces. The following is a list of acceptable special characters:
  - Underscore (_)
  - Period (.)
  - Dash (-)
  - Asterisk (*)

Upload sequence guidelines

Sequences can be uploaded to the design tool in a TXT file in FASTA format.

- The first line is called the header line. It must start with a greater-than symbol (>), and is followed by a unique identifier for the sequence. For example, the miRBase ID can be used as the unique identifier. For the assay name guidelines see, "Sequence design guidelines" on page 7.
- The second line contains the small RNA sequence.

Note: When uploading sequences from a TXT file, the header line becomes the assay name.

The text file can include the header and sequence for multiple assays.

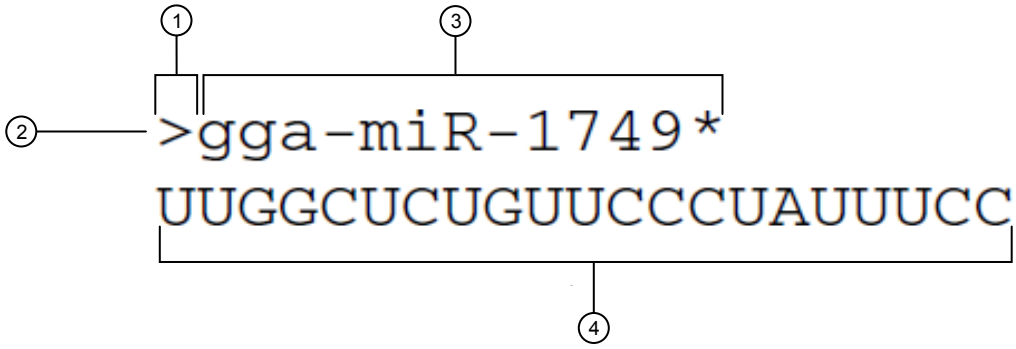


Figure 1 Custom assay header and sequence for one assay

1. Greater–than symbol
2. Unique identifier
3. Header line (used as the assay name)
4. Small RNA sequence

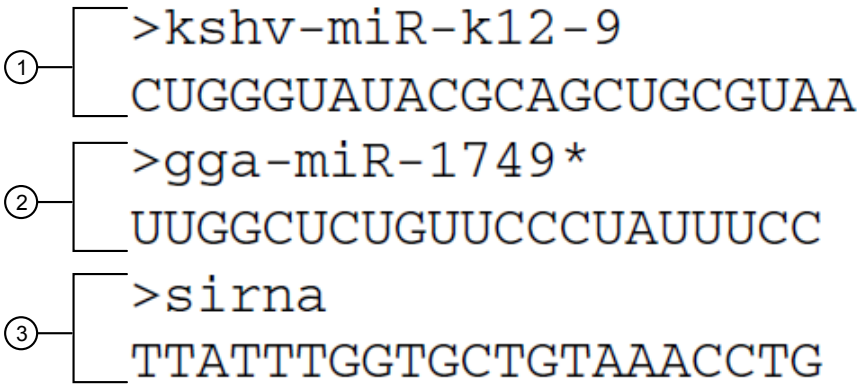


Figure 2 TXT file with header and sequence for multiple assays

1. Assay 1
2. Assay 2
3. Assay 3

Submit the sequences

Sequences can be entered manually in the design tool or uploaded in a TXT file in FASTA format.

Create a Thermo Fisher account

Go to **thermofisher.com** and create a Thermo Fisher account.

Upload sequences

To upload a TXT file, use the **Open/Import File** tool to find and select the file.

1. Sign in to your Thermo Fisher account.
2. Go to **thermofisher.com/taqmancustommirna**, then click **Order Custom TaqMan® Small RNA Assays**.
3. In the **Submit Sequences** tab of the Custom TaqMan® Small RNA Assays tool page, click **Open/Import File**.
4. Click **Choose File**, then select TXT file to upload.
5. Click **Import File**.
   The ***Assay Name*** and ***Sequence*** fields are filled with the selected sequence information.

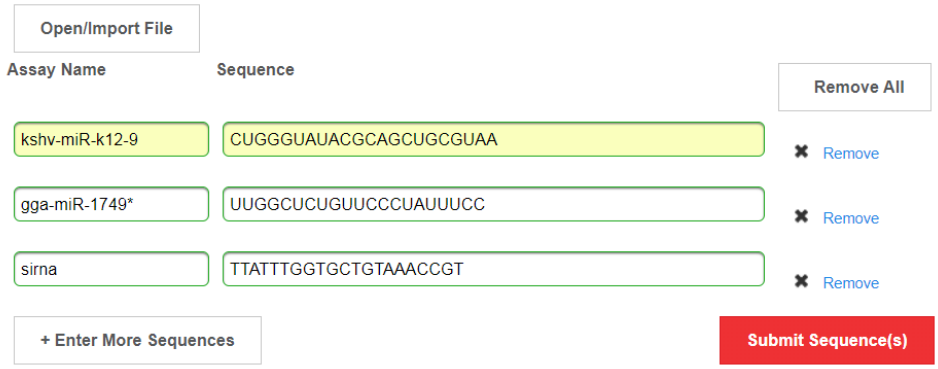


Figure 3 Example of uploaded sequences

6. Click **Submit Sequence(s)**.
   The design tool moves to the **Design Job Details** tab.
7. *(Optional)* Click **Refresh Batch List** to refresh the **Design Job Details** table.
8. *(Optional)* Close the design tool.
   Closing the design tool at this step will not result in a loss of your submission.

After submitting your sequences, the design tool sends a confirmation email to the address associated with your Thermo Fisher account. For more information see, "Email confirmation" on page 10.

Enter sequences manually

1. Sign in to your Thermo Fisher account.
2. Go to **thermofisher.com/taqmancustommirna**, then click **Order Custom TaqMan® Small RNA Assays**.
3. In the **Submit Sequences** tab of the Custom TaqMan® Small RNA Assays tool, type in the assay information into the ***Assay Name*** and ***Sequence*** fields.

   Note: Do not include the greater-than symbol (>) when entering assay names manually. Its only purpose is to indicate the beginning of the name in a FASTA batch file.

4. Click **Submit Sequence(s)**.
   The design tool moves to the **Design Job Details** tab.

5. *(Optional)* Click **Refresh Batch List** to refresh the **Design Job Details** table.

6. *(Optional)* Close the design tool.
   Closing the design tool at this step will not result in a loss of your submission.

After submitting your sequences, the design tool sends a confirmation email to the address associated with your Thermo Fisher account. For more information, see "Email confirmation" on page 10.

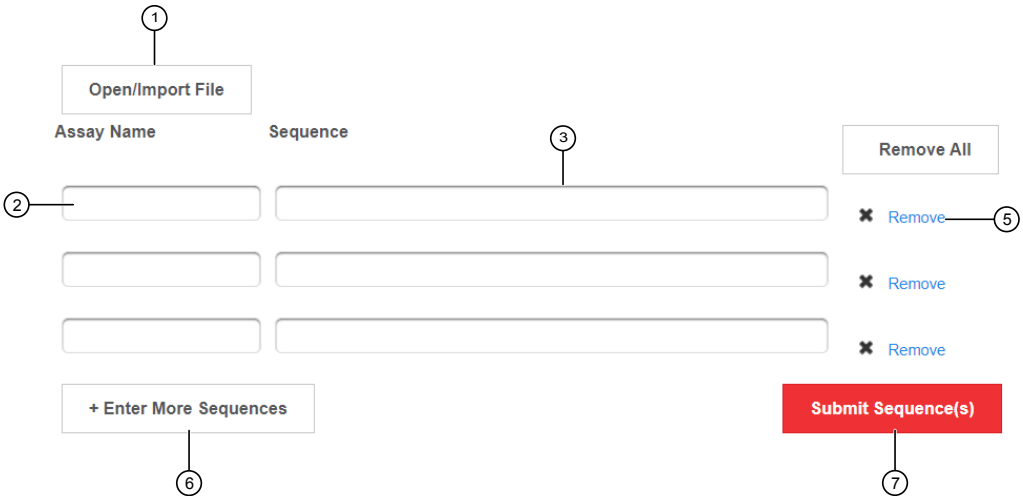


Figure 4 The **Submit Sequences** fields

① **Open/Import File** button—Opens the **FASTA File Import** dialog.
② ***Assay Name*** field—Enter the assay name here
③ ***Sequence*** field—Enter the sequence here
④ **Remove All** button—Deletes all entered sequences
⑤ **Remove** button —Deletes entered sequences individually
⑥ **+ Enter More Sequences** button—Adds additional rows for more assays
⑦ **Submit Sequence(s)** button—Completes the order

Email confirmation

After the sequences are submitted, the design tool will send a confirmation email to the address linked to your Thermo Fisher account. The email contains a link to the **Design Job Details** section of the design tool to view the status of your design job.

When the design job is complete, an email notification is sent with a link to view the results of your completed design job.

Select assays to order

The **Select Assays** tab displays assay information for each batch of submitted sequences. The assay information is divided into two tables **Design Job Details** and **Design Results**. For a complete description see, Appendix A, "About the design tables".

1. *(Optional)* If you closed the design tool, follow the link from the email notification to open the Custom TaqMan® Small RNA Assays tool.

2. Click a row in the **Design Job Details** table that corresponds to a submission of interest.
   The **Design results** tables for the selected row is displayed.
   If the **Custom Assay** table displays **DESIGN FAILED** see "Assay design failure" on page 12.

3. Review the list of custom assays and predesigned assays.

4. For each assay that you want to order, select the assay size from the dropdown list in the **Part Number and Size** column.
   The following options are available:
   - Extra small (XS)—75 PCR reactions
   - Small (SM)—150 PCR reactions
   - Medium (MED)—750 PCR reactions
   - Large (LG)—2,900 PCR reactions

5. Enter the quantity to order in the **Quantity** column.

6. Click **Add** next to an assay to add it to the **Shopping List** pane at the right edge of the screen.

7. *(Optional)* Click **Add All** to add all assays that are listed in the corresponding table to the **Shopping List**.

8. *(Optional)* Click the **Select Other Products** tab to order other required supplies.
   See "Select related products" on page 12

9. Click the green **Order Now** button in the **Shopping List** pane.
   The design tool opens the **Review & Order** tab.

Assay design failure

If the design for a TaqMan® Small RNA Assay fails, the **Design Job Details** table on the **Select Assays** tab shows **FAIL** in the **Status** column for the sequence. There are three possible error messages for failures.

- **Error Message 1**—We have not been able to design a high-performing assay for this sequence in the past. Contact technical support at **techsupport@thermofisher.com** to discuss purchasing an assay with relaxed performance standards.
- **Error Message 2**—The submitted sequence failed design. The design may have failed due to high G/C content, high A/T content, or the potential of the sequence to create self–dimers.
- **Error Message 3**—One or more of the assay oligonucleotides cannot be manufactured in a way that meets our high–performance standards. Contact technical support at **techsupport@thermofisher.com** to discuss whether it is possible to redesign the assay.

Select related products

The **Select Other Products** tab displays assays used to detect candidate endogenous controls for normalizing small RNA sequences submitted to the design tool. A valid normalization or endogenous control is needed to correct for differences in RNA sampling and sample variation. The ideal control is expressed consistently under experimental conditions and is sufficiently abundant across all tissues and cell types studied.

Note: We recommend that you experimentally verify candidate genes before using them as endogenous controls.

Add a control assay to the order

1. In the **Select Other Products** tab, review the list of control assays.
2. Click a species type to expand the list, then click the name of a control assay for details.
   - Human
   - Mouse
   - Rat
   - *A. thaliana*
   - *C. elegans*
   - *D. melanogaster*
3. To order an assay, fill in the **Quantity** column, then click **Add** to add it to the **Shopping List** pane.

Review and submit your order

1. In the **Shopping List** pane, click **Order Now**.
   The design tool opens the **Review & Order** tab.
2. Review your order.

3. *(Optional)* Enter a new quantity in the **Quantity** field.
4. *(Optional)* Select a new assay size from the dropdown list.
5. *(Optional)* To delete an assay, click the **x** next to the unwanted assay.
6. Click **Add To Cart**.
7. Complete the order as instructed by the website.
8. Click **Submit Order**.
   The design tool sends an email to confirm your order.
   The **Cart** window opens, and then displays the list of ordered items with the subtotals and total price.
9. Click **Begin checkout** to pay, then arrange shipping, or click **Transfer control of cart**.
   Transfer control of cart button assigns someone else to complete the transaction after you have ordered the required products.

Reorder assays

Assays can be reordered from the **Quick Order** page.

1. Log into your Thermo Fisher Scientific account.
2. Click **Quick Order**.
3. Enter the catalog number, quantities, and assay IDs in separate rows.
4. Click the **Cart** menu, then select an existing shopping cart to add the new items to, or create a new cart.
5. Click **Add to Cart**, then follow the instructions to complete the order.

About the design tables

Design Job Details table

The **Design Job Details** table displays the batches of sequences submitted through the design tool. Each row summarizes the design results for each batch.

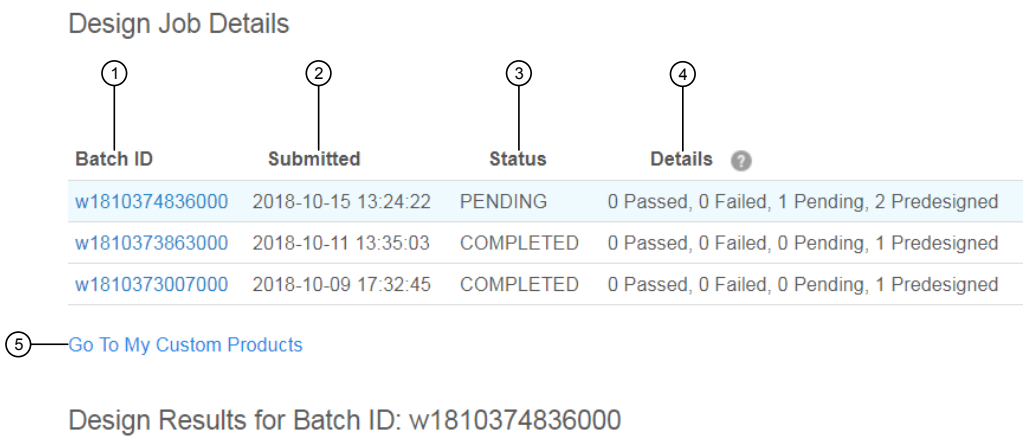


Figure 5 Design Job Details table

① **Batch ID**—Unique identifier automatically assigned
② **Submitted**—When the design job was submitted
③ **Status**—Job completed or pending
④ **Details**—Status of each sequence in a job
⑤ **Go To My Custom Products**—Displays previous custom assays

Design Results table

The Design Results table lists the TaqMan® Small RNA Assays that have been designed or found for each batch submitted. Select a row in the Design Job Details table to display the corresponding assays in the Design Results table.

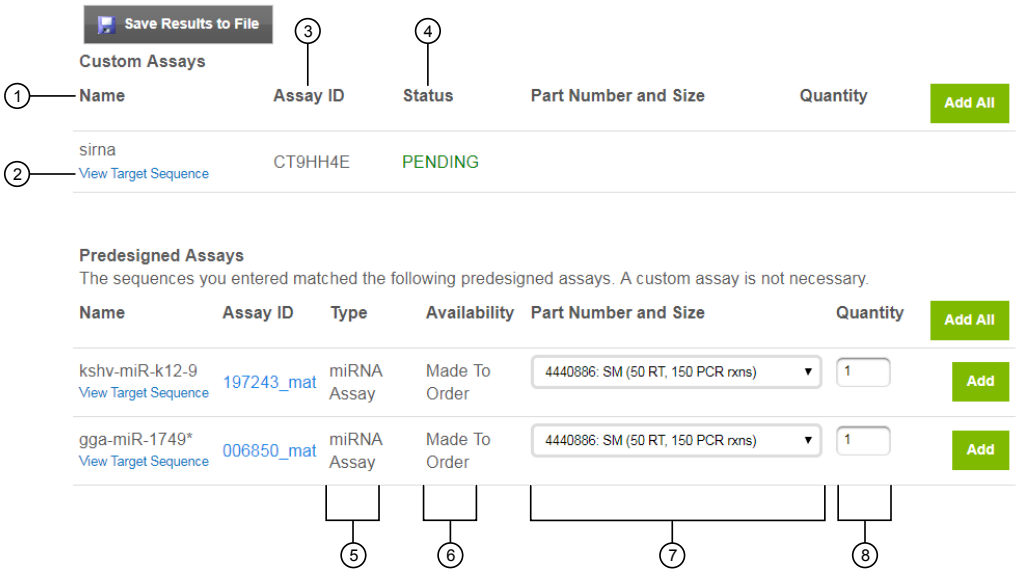


Figure 6 The **Design Results** table

1. **Name**
2. **View Target Sequence** button
3. **Assay ID**
4. **Status** (*Custom assays only*
5. **Type** (*Predesigned assays only*)
6. **Availability** (*Predesigned assays only*)
7. **Part Number and Size**
8. **Quantity**

Elements of Design Results table	Description
Name	User-entered sequence indentifier.
View Target Sequence button	Click or hover over to view the submitted sequence.
Assay ID	An automatically assigned identifier used for reordering.
Status (custom assays only)	Describes the status of a custom design. • **PENDING**— Assay design is still in progress. • **DESIGN SUCCESS**— Assay design is complete and the design tool was able to generate an optimal assay for the submitted sequence. • **DESIGN FAILED**— Assay design is complete, but the design tool was unable to generate an optimal assay for the submitted sequence. For more information see, "Assay design failure" on page 12.
Type (predesigned assays only)	Classification of the small RNA assay target (miRNA, siRNA, or other small RNAs).

Elements of Design Results table	Description
Availability (predesigned assays only)	• Made to order—The assay is manufactured at the time of order. • Inventoried—The assay in stock and ready to ship at the time of order.
Part Number and Size	A dropdown menu to select part number and size of assay to order.
Quantity	A field to enter the quantity of assay to order.

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).

- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Pub. No.
TaqMan® Small RNA Assay User Guide	4364031
TaqMan® Small RNA Assay Quick Reference	4412551
Custom Reverse Transcription Pools and Custom Preamplification Pools with TaqMan® MicroRNA Assays User Bulletin	4465407
Understanding Your Shipment	MAN0017153

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.