molecular probes®
by life technologies™

Click-iT® Nascent RNA Capture Kit

Table 1 Contents and storage

Material	Amount	Concentration	Storage*	Stability
5-ethynyl Uridine (EU, Component A)	5 mg	NA	• 2–6°C • Desiccate • Protect from light	When stored as directed, this kit is stable for at least 1 year.
Click-iT® EU buffer (Component B)	1 mL	2X		
Biotin azide (PEG_4 carboxamide-6-azidohexanyl biotin) (Component C)	340 µg	NA		
Copper (II) Sulfate ($CuSO_4$) (Component D)	200 µL	25 mM aqueous solution		
Click-iT® reaction buffer additive 1 (Component E)	400 mg	NA		
Click-iT® reaction buffer additive 2 (Component F)	100 µL	NA		
Click-iT® RNA binding buffer (Component G)	2 × 1 mL	2X		
Dynabeads® MyOne™ Streptavidin T1 (Component H)	500 µL	NA		
Click-iT® reaction wash buffer 1 (Component I)	35 mL	1X		
Click-iT® reaction wash buffer 2 (Component J)	35 mL	1X		

*These storage conditions are appropriate when storing the entire kit upon receipt. After preparing stock solutions, optimal storage conditions may change. For storage conditions of individual kit components, see the product labels. For storing prepared stock solutions, follow the recommendations included in this product information sheet. NA = Not applicable.

Number of assays: Based on the protocols below, the kit contains material for 6 to 40 RNA capture reactions depending on the type, abundance, transcription kinetics, turnover of the particular RNA species. For each RNA capture reaction, up to 10 post-capture analysis assays can be performed. We recommend that the users familiarize themselves with the protocol and amount of reagents provided in the kit to match with their experimental requirements. For the recommended EU concentrations and labeling volumes used in various labeling reaction formats, see Table 2.

Introduction

Changes in the transcriptome are fundamental to homeostasis, differentiation, and disease. Monitoring the changes in transcripts by determining their sequence, copy number, and half-life are necessary for accurately characterizing the dynamics of the transcriptome. The Click-iT® Nascent RNA Capture Kit facilitates the partitioning of the newly synthesized RNA transcripts from the already existing RNA. This approach effectively allows a high resolution investigation of the transcriptome as the cell or the tissue undergoes changes. Using this approach, information about the RNA sequence, copy number, and half life can be obtained.

A major advantage of the Click-iT® Nascent RNA Capture Kit is that it requires no radioactive nucleoside analogs such as those used in nuclear run-on, run-off experiments. The Click-iT® Kit facilitates the study of RNA stability, RNA synthesis, RNA decay, and transcriptional regulation, and enables more sensitive and accurate ΔΔC(t) measurements. The captured transcripts can be used as a template for reverse transcriptase-mediated cDNA synthesis for subsequent analysis using qPCR (SYBR® Green or TaqMan® assays). This cDNA can also be used for arrays such as TaqMan® low density arrays (TLDA), open arrays, microarrays, as well as RNA and DNA sequencing on NextGen SOLiD® or Illumina® instruments.

The Click-iT® Nascent RNA Capture protocol (Figure 1) begins with the incubation of live cells with an analog of uridine, 5-ethynyl uridine (EU, an alkyne-modified nucleoside), which is efficiently and naturally incorporated into the nascent RNA. After the incubation, total RNA or mRNA labeled with EU is isolated and used in a copper catalyzed click reaction with an azide-modified biotin (Figure 2), which creates a biotin-based handle for capturing nascent RNA transcripts on streptavidin magnetic beads. The EU has been shown to be non-toxic to the cells as evidenced by propidium iodide/Annexin staining (see Figure 3) and it does not affect the global transcriptome of the cell. In a microarray analysis of over 17,859 genes, we could only find minor changes to 4 genes compared to the control vehicle (Figure 4), with no effects on cell proliferation. In addition, EU is water-soluble and thus compatible with most physiological buffers.

Click reactions have several general characteristics: the reaction is efficient, no extreme temperatures or solvents are required, the reaction is complete within 30 minutes, the components of the reaction are bio-inert, and perhaps most importantly, no side reactions occur—the label and detection tags react selectively and specifically with one another.[1–4] This final point is a key advantage of this powerful detection technique; it is possible to apply click chemistry-labeled molecules to complex biological samples and detect them with unprecedented sensitivity due to the extremely low background of the reaction. The Click-iT® Nascent RNA Capture Kit contains all of the components needed to label and capture newly synthesized RNA transcripts in as few as 25,000 cells in a variety of labeling volumes (see Table 2). Based on the protocols below, the kit contains material for 6 to 40 RNA capture reactions depending on the type, abundance, transcription kinetics, turnover of the particular RNA species. For each RNA capture reaction, up to 10 post-capture analysis assays can be performed. We recommend that the users familiarize themselves with the protocol and amount of reagents provided in the kit to match with their experimental requirements. For the recommended EU concentrations and labeling volumes used in various labeling reaction formats, see Table 2. For larger quantities, inquire at www.lifetechnologies.com.

Table 2 EU concentration and reaction volume

Culture vessel	EU concentration	Quantity	Reaction volume
12-well plate	0.5 mM	3 plates	1 mL per well
6-well plate	0.5 mM	2 plates	3 mL per well
	0.2 mM	5 plates	
	0.1 mM	10 plates	
100-mm dish	0.2 mM	9 dishes	10 mL
T75 flask	0.2 mM	6 flasks	15 mL
T175 flask		2 flasks	35 mL

Note: For 40 reactions, use an EU concentration of 0.4 mM into a 12-well plate.

Figure 1 Workflow for the Click-iT® Nascent RNA Capture

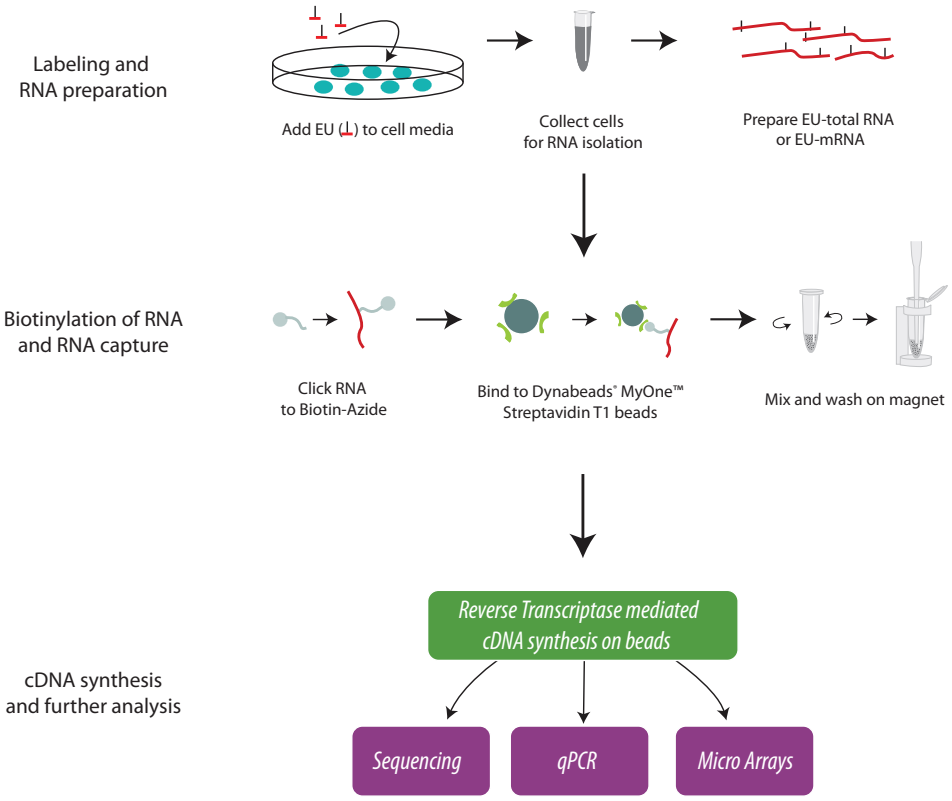


Figure 2 Click reaction between EU and azide-modified dye or biotin

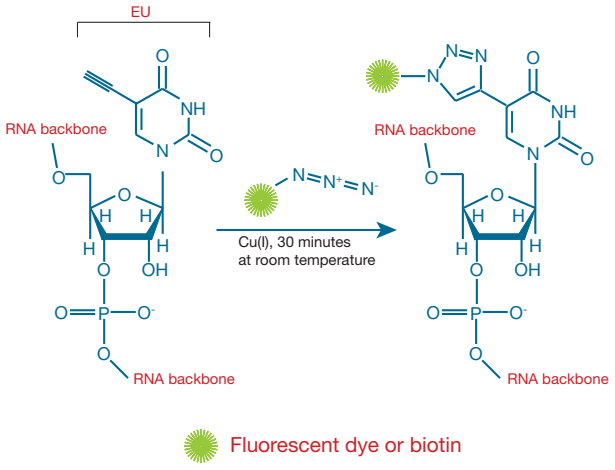


Figure 3 Feeding of EU to cultured cells does not cause any apoptosis or unnatural cell death as determined by flow cytometry. Jurkat cells were fed 200 µM EU for 4 hours and the cell toxicity was determined using Alexa Fluor® 488 Annexin V/propidium iodide stain (Cat. no. V13241). The results showed no differences in live, apoptotic or dead cell populations between EU treated and control.

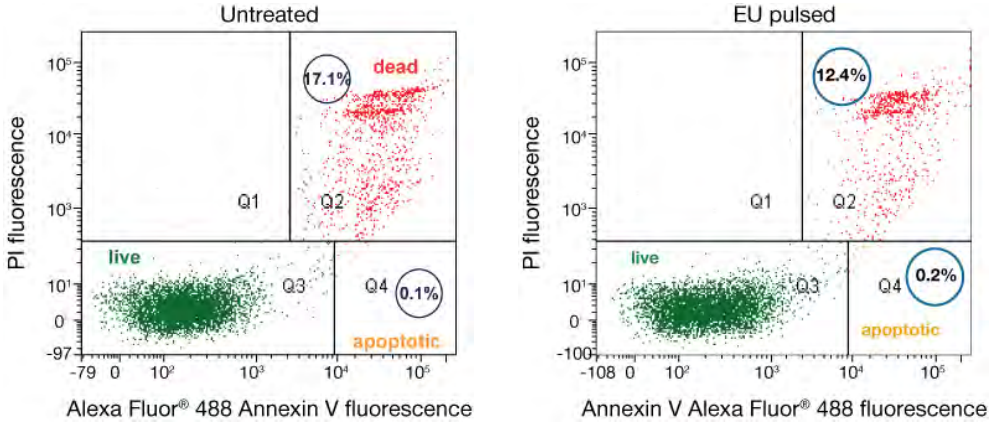
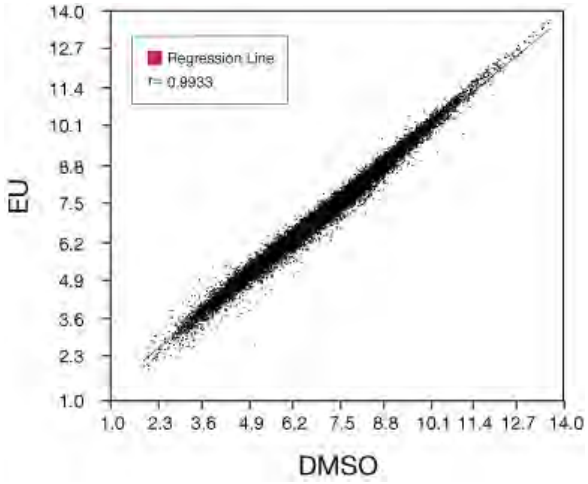


Figure 4 Effect of EU incorporation on the global transcriptome was analyzed by microarray analysis. A549 cells were fed 200 µM EU for 4 hours while the control was treated with DMSO. Microarray analysis was carried out using the Ambion® WTEK kit and the Affymetrix fragmentation and labeling kit on an Affymetrix® GeneChip Human Exon 1.0 ST array. Partek® Genomics Suite™ software was used to process the data using standard RMA normalization and perform statistical tests. The data showed no differences between EU treated and control cells.



Before starting

Materials required but not provided

- RNAseOUT™ Recombinant Ribonuclease Inhibitor (Cat. no. 10777-019)
- UltraPure™ DNase/RNase-Free Distilled Water (Cat. no. 10977-023)
- DynaMag™-2 Magnet (Cat. no. 12321D) or DynaMag™-Spin Magnet (Cat. no. 12320D)
- SuperScript® VILO™ cDNA synthesis kit (Cat. no. 11754-050)
- UltraPure™ Glycogen (Cat. no. 10814-010)
- 7.5 M ammonium acetate
- 100% ethanol (i.e., absolute ethanol), chilled
- 75% ethanol

Caution

- DMSO is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO (e.g., EU stock solution in DMSO) using equipment and practices appropriate for the hazards posed by such materials. Dispose off the reagents in compliance with all pertaining local regulations.
- Copper (II) Sulfate ($CuSO_4$) (Component D) is harmful to aquatic organisms, and it may cause long-term adverse effects in the aquatic environment. Dispose of the reagent in compliance with all pertaining local regulations.
- Click-iT® RNA binding buffer (Component G) contains tetramethylammonium chloride, which is toxic if swallowed. Handle the Click-iT® RNA binding buffer using equipment and practices appropriate for the hazards posed by such materials.

Experimental protocols

Preparing stock solutions

1.1 To prepare 200 mM EU stock solution, dissolve the entire contents (5 mg) of the EU vial (Component A) in 93 µL of DMSO or DNase/RNase-free distilled water. Mix the solution vigorously by vortexing. The solution is dark brown in color. Store the solution at –20°C until further use.

1.2 To prepare 10 mM biotin azide stock solution, dissolve the entire contents (340 µg) of the biotin azide vial (Component C) in 55 µL of DMSO. Vortex the solution to mix. Store the solution at –20°C, in the dark, until use.

1.3 To make a 400 mM Click-iT® reaction buffer additive 1 stock solution, dissolve the entire contents (400 mg) of the Click-iT® reaction buffer additive 1 (Component E) vial in 5 mL of DNase/RNase-free distilled water. Aliquot the solution into five 1 mL or ten 0.5 mL fractions. Store the aliquots at –20°C, in the dark. You may freeze and thaw the solution repeatedly, but do not leave the solution at room temperature overnight. Discard the solution if it begins to turn yellow.

Labeling the cells with EU

- The following protocol was developed with Jurkat, HeLa, A549, and HEK293 cells, seeded at 40–50% confluency. Typical Jurkat cell density was between 0.5 to 1 million per mL at the time of EU pulsing.
- The following protocol is based on testing 6 conditions by growing the cells in six T75 flasks (i.e., one condition per flask) containing 15 mL of cell culture medium each, pulsing the cells with 0.2 mM EU, and then capturing the total EU-labeled RNA. See Table 2 for capture assays performed in other reaction formats and volumes.
- Growth medium, cell density, cell type variations, and other factors may influence the assay conditions. In initial experiments, we recommend testing a range of EU concentrations to determine the optimal concentrations for your cell type and experimental conditions. Table 3 lists the recommended EU doses and incubation time for initial experiments.

Table 3 Recommended EU doses and incubation time

EU concentration	Recommended time for pulse labeling
0.5 mM	0.5 to 1 hour
0.2 mM	1 to 24 hours
0.1 mM	

- Additional EU (Cat. no. E10345) and biotin azide (Cat. no. B10184) are available separately from Life Technologies.

2.1 Add the appropriate volume of EU stock solution (prepared in Step 1.1) to the culture medium containing the cells. For example, add 15 µL of 200 mM EU stock solution to 15 mL of suspension cells in a T75 flask for a final EU concentration of 0.2 mM. Make sure that the EU solution is well dispersed by moving the culture vessel back-to-front and side-to-side.

Note: For 40 reactions with a reaction volume of 1 mL, use 1 µL/well in a 12-well plate.

Note: You may also prepare a 2X working solution of EU from the 200 mM stock solution (prepared in Step 1.1) in **pre-warmed** complete medium. For example, for a 0.2 mM final EU treatment, prepare a 0.4 mM working solution by diluting the stock solution 1:500 in pre-warmed complete medium. Add an equal volume of this 2X EU working solution to the media containing cells.

2.2 Incubate the cells at 37°C at 5% CO_2 for 1 to 24 hours, as needed. You may use higher final concentrations of the EU solution (e.g., 0.5 mM or 1 mM) for shorter pulses ranging from 20 minutes to 1 hour. The duration of pulsing depends on the condition and objective of the experiment and the cell type in use.

2.3 Harvest the cells and transfer an appropriate amount into sterile centrifuge tubes. Centrifuge the tubes at 250 × *g* for 5 minutes. Aspirate off the culture medium and proceed to RNA preparation.

2.4 *Optional*: Snap freeze the cell pellet in liquid nitrogen for 15 seconds and store the cells at –80°C until further use.

Note: This is a break point in the experiment. You may store the cells at –80°C until further use.

Preparing RNA for Click reaction

Preparing total RNA

We have routinely and successfully used the TRIzol® reagent (Cat. no. 15596-018) for preparing total RNA following the protocol provided with the reagent.

Preparing mRNA

We have routinely prepared high quality mRNA using the Poly(A)Purist™ mRNA purification kit (Cat. no. AM1916) following the instructions provided with the kit.

Transcriptome RNA purification

The RiboMinus™ kits use a novel purification technology that enriches the whole RNA transcript spectrum by selective depletion of rRNA transcripts from total RNA. The kits are available for enrichment of transcriptome RNA from human/mouse, yeast and bacteria. For more information, refer to www.lifetechnologies.com.

Note: This is a break point in the experiment. You may store the total or mRNA at –20°C until further use.

Biotinylation of RNA by Click reaction

The total RNA concentration for the click reaction should be 1 µg/µL. The total amount of RNA used depends on the type, abundance, turnover, half life, and method of preparation of the RNA, as well as the length and concentration of the EU pulse. Refer to Table 4, below, for the recommended ratio of EU-labeled RNA (EU-RNA) to the biotin azide working solution.

Table 4 Recommended EU-RNA and biotin azide ratio

EU-RNA	Biotin Azide
10 µg	1 mM
5 µg	0.5 mM
1 µg	0.5 mM
500 ng	0.25 mM

3.1 Thaw the 10 mM Biotin azide and an aliquot of the Click-iT® reaction buffer additive 1 stock solution (prepared in steps 1.2 and 1.3, respectively) at room temperature.

3.2 Prepare the Click-iT® reaction cocktail (50 µL per reaction) according to Table 5, page 8. Add the reaction components in the order listed, following the guidelines below:

- After adding each component, mix the reaction cocktail **gently** by vortexing it briefly or by pipetting the solution up and down.
- After adding the Click-iT® reaction buffer additive 1 stock solution (prepared in Step1.3), immediately mix the reaction cocktail by **gently** pipetting it up and down. This step initiates the click reaction between the EU-RNA and biotin azide.
- Add the Click-iT® reaction buffer additive 2 (Component F) **3 minutes** after adding the Click-iT® reaction buffer additive 1 stock solution. The reaction mix should turn dark brown upon addition of the Click-iT® reaction buffer additive 2.

Table 5 Click-iT® reaction cocktail (50 µL total reaction volume)

Order of addition	Reaction component	Stock concentration	Final concentration	T-75 flask 6 reactions 50 µL per reaction	12-well plate 40 reactions 50 µL per reaction
1	Water	–	–	variable*	variable*
2	Click-iT® EU buffer (Component B)	2X	1X	25 µL	25 µL
3	$CuSO_4$ (Component D)	25 mM	2 mM	4 µL	4 µL
4	Biotin azide (Component C)	10 mM	0.25–1 mM	5 µL	1.25 µL
5	EU-RNA	–	–	variable*	variable*
6	Click-iT® reaction buffer additive 1 (Component E)	400 mM	10 mM	1.25 µL	1.25 µL
7	Click-iT® reaction buffer additive 2 (Component F)	400 mM	12 mM	1.5 µL	1.5 µL

*The volume per reaction depends on the concentration of EU-RNA.

Note: The timing for adding the Click-iT® reaction buffer additive 2 is critical, and it must be followed as closely as possible.

3.3 Incubate the click reaction mix for 30 minutes by **gently** vortexing it on a laboratory vortex mixer.

RNA precipitation

4.1 Add 1 µL of UltraPure™ Glycogen, 50 µL of 7.5 M ammonium acetate, and 700 µL of chilled 100% ethanol to the click reaction. Mix the tube contents by inverting the tube several times or by gently vortexing it.

4.2 Incubate the tube overnight at –70°C (recommended) or on dry ice or at –70°C for 30 minutes.

4.3 Centrifuge the tube at 13,000 × g for 20 minutes at 4°C.

4.4 Remove the supernatant from the tube without disturbing the pellet and add 700 µL of 75% ethanol. Vortex the tube briefly and centrifuge it at 13,000 ×g for 5 minutes.

4.5 Repeat Step 4.4, let the pellet dry for 5 to 10 minutes at room temperature, and resuspend it in 50 µL of Ultra-Pure™ DNase/RNase-free distilled water.

4.6 Measure the RNA concentration using a spectrophotometer. For best results, we recommend using the Qubit™ fluorometer or a UV-Vis spectrophotometer (e.g., NanoDrop™). Typical RNA yield after this procedure is approximately 90% of the starting material.

Note: This is a break point in the experiment. You may store the resuspended RNA at –20°C until further use.

Binding biotinylated RNA to Dynabeads® MyOne™ Streptavidin T1 magnetic beads

For the recommended amounts of biotinylated RNA and magnetic beads to use in the binding reaction, refer to Table 6, below.

Table 6 Recommended amounts of biotinylated RNA and magnetic beads to use in the binding the reaction

Amount of biotinylated RNA	Volume of magnetic beads per reaction
0.5–1 µg	50 µL
250–400 ng	25 µL
100–200 ng	15 µL
75–100 ng	10 µL
25–50 ng	5 µL

We typically use 50 µL of Dynabeads® MyOne™ Streptavidin T1 magnetic beads per 500 ng of biotinylated total RNA. For general guidelines on how to handle and use the magnetic beads, refer to the product information sheet supplied with the Dynabeads® MyOne™ Streptavidin T1 magnetic beads (Cat. no. 656-01), which is available for downloading at www.lifetechnologies.com.

Note: All the buffers necessary to use the magnetic beads with the Click-iT® Nascent RNA Capture Kit are included in the kit. Do **not** use the buffers recommended in the product information sheet supplied with the magnetic beads for nascent RNA capture using the Click-iT® Nascent RNA Capture Kit.

The following protocol is based on performing six different RNA capture reactions, using 50 µL of magnetic beads and 250 µL of RNA binding reaction mix per reaction (300 µL total reaction volume) or 40 different RNA capture reactions, using 12 µL of magnetic beads and 62 µL of RNA binding reaction mix per reaction (74 µL total reaction volume).

5.1 Centrifuge the Dynabeads® MyOne™ Streptavidin T1 (Component H) and remove the resulting supernatant.

5.2 Wash the beads 3X with 500 µL of Click-iT® reaction **wash buffer 2** (Component J), following the washing procedure outlined in the product information sheet supplied with the magnetic beads. This product information sheet is available for downloading at www.lifetechnologies.com.

Note: Make sure that you use the Click-iT® reaction wash buffer 2 (Component J) for the wash, and **not** the Click-iT® reaction wash buffer 1 (Component I).

5.3 After the final wash, add 500 µL of Click-iT® reaction **wash buffer 2** (Component J) along the inside of the tube where the beads are collected to resuspend the beads in the same volume of wash buffer 2 as the initial volume of Dynabeads® taken from the vial.

5.4 Prepare RNA binding reaction mix per reaction according to Table 7, below.

Table 7 RNA binding reaction mix (per reaction)

Component	Volume (6 reactions)	Volume (40 reactions)
Click-iT® RNA binding buffer, 2X (Component G)	125 µL	31 µL
RNAseOUT™ Recombinant Ribonuclease Inhibitor	2 µL	2 µL
RNA (from Step 4.6)	0.5–1 µg	0.5–1 µg
UltraPure™ DNase/RNase-Free Distilled Water	to 250 µL	to 62 µL

Note: The total volume of the RNA binding mix below is sufficient for a single binding reaction; for more reactions, scale the volumes accordingly.

5.5 Heat the RNA binding reaction mix (prepared in Step 5.4) at 68–70°C for 5 minutes.

5.6 Add the volume of bead suspension (washed in Step 5.2) indicated in Table 8 into each of heated RNA binding reaction mixes (see Step 5.4).

Table 8 Recommended volumes of bead suspension and wash buffers

Number of reactions	Bead suspension	Component I (wash buffer 1)	Component J (wash buffer 2)	Resuspension volume
6 reactions	50 µL	500 µL	500 µL	50 µL
40 reactions	12 µL	120 µL	120 µL	12 µL

5.7 Incubate the microcentrifuge tubes containing the RNA binding reactions at room temperature for 30 minutes while gently vortexing them on a laboratory vortex mixer to prevent the beads from settling.

5.8 Immobilize the beads using the DynaMag™-2 magnet or the DynaMag™-Spin magnet, and wash them 5X with 10-fold the volume of beads used in Step 5.6 (Table 8) with Click-iT® reaction **wash buffer 1 (Component I)**.

5.9 Wash the immobilized beads 5X with 10-fold the volume of beads uses in Step 5.6 (Table 8) with Click-iT® reaction **wash buffer 2 (Component J)**.

5.10 Resuspend the beads in the same volume of Click-iT® reaction wash buffer 2 (Component J) used in Step 5.6 (Table 8).

Note: At this point, the beads are ready to be used in a cDNA synthesis reaction. We highly recommend that you **immediately** use the RNA captured on the beads as a template for cDNA synthesis or use the RNA for the desired downstream analysis. Do **not** store the RNA for future use.

5.11 Proceed **immediately** to cDNA synthesis using the RNA captured on the beads as a template.

cDNA synthesis for RT qPCR

Use the RNA bound to the beads (from Step 5.10) as a template for cDNA synthesis. We recommend using the SuperScript® VILO™ cDNA synthesis kit (Cat. no. 11754-050). The following protocol has been optimized for generating first-strand cDNA for use in two-step RT qPCR. Table 9, below, lists the reaction components and their recommended volumes for a 100 µL cDNA synthesis reaction.

Table 9 cDNA synthesis reaction

Component	Volume
5X VILO™ Reaction Mix	20 µL
10X SuperScript® Enzyme Mix	10 µL
RNA, captured on beads (from Step 5.10)	50 µL
UltraPure™ DNase/RNase-Free Distilled Water	20 µL

6.1 Heat the bead suspension for 5 minutes at 68–70°C for 5 minutes.

6.2 Immediately add 20 µL of 5X VILO™ reaction mix into each bead suspension.

6.3 Bring the bead suspension-5X VILO™ reaction mixture to ambient temperature while gently mixing (e.g., on a slow vortex for 5–10 minutes). This allows the random primers in the VILO™ reaction mixture to anneal to the template RNA.

6.4 Add 20 µL of UltraPure™ DNase/RNase-Free Distilled Water to the reaction mixture.

6.5 Add 10 µL of 10X SuperScript® Enzyme mix to the bead suspension-5X VILO™ reaction mixture. Mix the reaction well by pipetting it up and down.

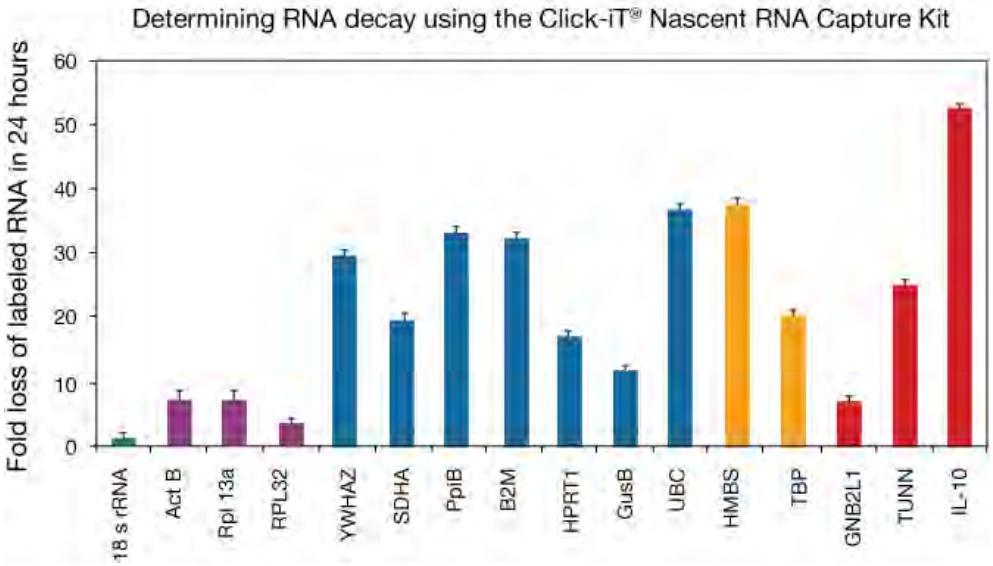
6.6 Incubate the reaction at 42°C to 50°C for 1 hour while gently mixing it. It is crucial that the reactions are gently mixed during the RT reaction to prevent the beads from settling.

6.7 Heat the reaction mixture at 85°C for 5 minutes to terminate the RT reaction and to release the cDNA from the beads.

6.8 Use the diluted or undiluted cDNA in qPCR (see below), or store it at –20°C until further use. You may use DynaMag™-2 magnet (Cat. no. 12321D) or the DynaMag™-Spin magnet (Cat. no. 12320D) to immobilize the beads while collecting the supernatant containing the cDNA.

Note: This is a breakpoint in the experiment. You may store the cDNA at –20°C until further use, or proceed to your downstream application such analysis using qPCR (SYBR® Green or TaqMan® assays) (Figure 5, page 12). This cDNA can also be used for arrays such as TaqMan® low density arrays (TLDA), open arrays, microarrays, as well as RNA and DNA sequencing on NextGen SOLiD® or Illumina® instruments.

Figure 5 The Click-iT® RNA capture method represents a novel systemic approach to simultaneously analyze short term changes in RNA synthesis and decay and their impact on cellular transcript levels. The traditional pulse chase experiments are used to measure the loss of labeled RNA to determine RNA decay or half-life. In this case, Jurkat cells were pulsed with EU for 24 hours and the medium was replaced with growth medium without EU (chase). Total RNA was isolated and subjected to nascent RNA capture using the Click-iT® Nascent RNA Capture Kit. RT-qPCR analysis was performed using standard protocols. The figure shows the loss of labeled RNA after the 24 hour chase. The RNA have been arranged in decreasing order of relative abundance, and are grouped into sets by color. Alternatively, one can vary the time of EU pulsing followed by nascent RNA capture to understand the kinetics of synthesis and estimate the turnover by dividing the C(t) value of nascent RNA by the total RNA (data not shown).



qPCR using SYBR® Green or SYBR® GreenER™

If you started with 500 ng or 1 µg total RNA, up to 10% of the qPCR reaction volume may be undiluted cDNA (e.g., for a 20 µL qPCR, use up to 2 µL of undiluted cDNA). We recommend that you prepare several dilutions of your cDNA to get a quantitative determination of the nascent RNA copy number.

References

1. Chem Bio Chem 4, 1147 (2003); **2.** J Am Chem Soc 124, 3192 (2003); **3.** Angew Chem Int Ed Engl 41, 2596 (2002); **4.** Angew Chem Int Ed Engl 40, 2004 (2001).

Product list Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product name	Unit Size
C10365	Click-iT® Nascent RNA Capture Kit *for gene expression analysis*	1 kit
Related products		
123-20D	DynaMag™-Spin magnet	each
123-21D	DynaMag™-2 magnet	each
656-02	Dynabeads® MyOne™ Streptavidin T1 Magnetic Beads	10 mL
10777-019	RNAseOUT™ Recombinant Ribonuclease Inhibitor	5,000 units
10814-010	UltraPure™ Glycogen	100 µL
10977-023	UltraPure™ DNase/RNase-Free Distilled Water	10 × 500 mL
11754-050	SuperScript® VILO™ cDNA synthesis kit	50 × 20 µL
15596-018	TRIzol® Reagent	200 mL
AM1916	Poly(A)Purist™ mRNA purification kit	6 purifications
B10184	biotin azide	1 mg
E10345	5-ethynyl uridine (EU)	5 mg
V13241	Vybrant® Apoptosis Assay Kit #2 *Alexa Fluor® 488 annexin V/propidium iodide* *50 assays*	1 kit

Purchaser notification

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Additional international offices are listed at www.lifetechnologies.com

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining Support
For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

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- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS
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