C-type RT Activity Kit

INSTRUCTIONS FOR USE

For Research Purposes Only

Cat# 51020

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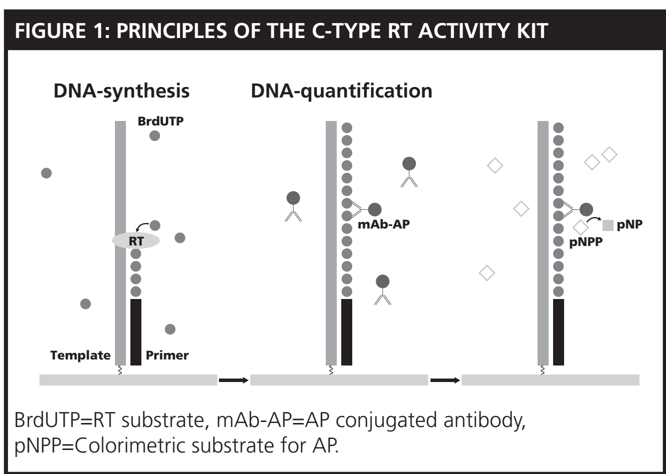
Intended Use

The C-type RT Activity kit is intended for detection or quantification of the activity of the enzyme Reverse Transcriptase (RT) from C-type retroviruses, like Murine leukemia virus (MuLV), Porcine endogenous retrovirus (PERV), Feline leukemia virus (FeLV) and Spumavirus.

Principles of the C-type RT Activity Kit

The C-type RT Activity kit determines the RT activity in a sample. The procedure consists of two steps, the DNA synthesis and the DNA quantification. The principle is illustrated in figure 1. The figure shows a well in the 96-well Poly A Plate with the RNA template bound to the bottom.

FIGURE 1: PRINCIPLES OF THE C-TYPE RT ACTIVITY KIT



BrdUTP=RT substrate, mAb-AP=AP conjugated antibody, pNPP=Colorimetric substrate for AP.

A reaction mixture, containing primer and a nucleotide (BrdUTP), is added to the plate as well as the sample to be analysed. The RT in the sample will synthesise a DNA-strand. An alkaline phosphatase (AP) conjugated α-BrdU antibody binds to the double stranded DNA/RNA molecule. The product is quantified by addition of a colorimetric AP substrate. The AP activity is proportional to the RT activity in the sample.

Applications

The C-type RT Activity kit instructions for use describes two kit applications, quantification and screening for RT activity. The measuring range of the assay is 0.04 to 100 mU/ml*.

PROTOCOL A: QUANTIFICATION OF RT ACTIVITY

MAXIMUM NUMBER OF SAMPLES ANALYSED IN ONE KIT: 20 (40**)

TYPE OF SAMPLE: Cell culture supernatant, cell extracts or serum SAMPLE VOLUME: 40 µl

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When analysing crude samples, RT inhibitory factors may be present. To ensure a quantitative determination in such samples, it is important to establish that the enzyme reaction is linear with time. It is also important to verify that the measured RT activity is proportional to the concentration of the sample in the assay. Thus samples to be quantified should be analysed at several dilutions as well as at different RT reaction times**. The level of RT activity in the sample is calculated from the MMuLV rRT Standard set on each plate.

PROTOCOL B: SCREENING FOR RT ACTIVITY

MAXIMUM NUMBER OF SAMPLES ANALYSED IN ONE KIT: 80 (160**)

TYPE OF SAMPLE: Cell culture supernatant, cell extracts or serum SAMPLE VOLUME: 10 µl + 10 µl

When processing a large series of samples, of which only a few are expected to be RT positive, it may be advantageous to perform a semi-quantitative screening assay. An example is when analysing cell supernatants from virus isolation cell cultures for presence of MMuLV.

Protocol B differs from Protocol A in that a small amount of crude undiluted sample is used. The small sample volume is necessary to avoid disturbances.

*The measuring range is based on the calculations described in the Data processing section on page 9, using the included *MMuLV rRT Standard* as reference enzyme. It can, however, be improved further by using a reference wavelength of 630 nm or using kinetic readings.

**Once the system is standardised for your application there is no need for analysis with different RT reaction times. The kit can then be used for twice as many samples. If you need to use the two *Poly A Plates* at different occasions, prepare a new *MMuLV rRT Standard* for each occasion (two vials included with each kit).

Analysing other RT Isozymes

The C-type RT Activity kit contains a recombinant MMuLV RT as reference enzyme. The kit may, however, be used for other RT isozymes than MMuLV. For this purpose it is recommended to use an aliquoted and frozen (-70°C) in-house RT standard of the same isozyme type. The standard may be used instead of, or in parallel with, the *MMuLV rRT Standard.*

Prepare a serial dilution of the in-house reference enzyme in the same manner as described for the *MMuLV rRT Standard* in protocols A or B. The amount RT in the first step of the dilution series must be standardised to give an activity in the same range as the *MMuLV rRT Standard.*

Components

KIT COMPONENTS

2 *Poly A Plate* (96 wells)
1 *C-type Sample Dilution Buffer* (30 ml)
2 *C-type Sample Dilution Components* (lyophilised)
2 *C-type RT Reaction Components* (lyophilised)
1 *C-type Reconstitution Buffer* (25 ml)
2 *MMuLV rRT Standard* (lyophilised)
1 *C-type Plate Wash Buffer conc.* (50 ml)
2 *C-type RT Product Tracer* (lyophilised)
1 *Substrate Tablet* (15 mg)
1 *Substrate Buffer* (30 ml)
4 Pieces of Adhesive Tape
2 Plastic Lids for 96-well plate
1 MMuLV rRT Standard Sheet for C-type RT Activity Kit

EQUIPMENT REQUIRED BUT NOT PROVIDED

Purified water
Triton X-100 (laboratory grade or higher)
Magnetic spinner and spin bars
Incubator set at 33°C
ELISA-plate reader with A_{405} filter
ELISA-plate washer or 4 wash buckets (approx. 3 litre)
Orbital shaker suitable for ELISA-plates
Vortex
Single-channel pipettes and tips (10-1000 µl)
Multi-channel pipettes and tips (10-200 µl)
Reservoirs for multi-channel pipettes
Measuring cylinders, pipettes etc.
Bottles for 50 ml and 2 litres
Container for 10 litres
12 Test tubes (about 1 ml)
1 Standard 96-well microtitre plate with 300-µl wells for titrations (For protocol A only)

DESCRIPTION OF COMPONENTS

- The *Poly A Plate* has pr(A) (polyriboadenosine) strands covalently bound to the wells.
- The *C-type RT Reaction Components* contains lyophilised odT (oligodeoxythymidine) primer and BrdUTP (5-bromo-3-deoxyribouridine 5'-triphosphate).
- The *MMuLV rRT Standard* contains lyophilised recombinant MMuLV RT.
- The *C-type RT Product Tracer* contains lyophilised monoclonal α-BrdUMP (anti bromo-deoxyribouridine monophosphate) antibodies conjugated to alkaline phosphatase.
- The *Substrate Tablet* contains pNPP (paranitrophenylphosphate disodium).

Kit Storage and Shelf Life

- Store the kit reagents at between -14 and -25°C until use.
- If delivered unfrozen, the kit should be refrozen and stored between -14 and -25°C or stored at max. 8°C for usage within a week.
- Once reconstituted, lyophilised components should not be refrozen, but can be stored at 4 to 8°C for usage within a week.

Sample Collection, Preparation and Storage

CELL CULTURE SUPERNATANTS for RT analysis should be sampled carefully to avoid transferring cells. For repeated analysis or later experiments, duplicate samples may be aliquoted and frozen at -20°C. It is recommended that the frozen samples are used within two weeks. For longer storage, keep the samples at -70°C.

CELL EXTRACTS may contain substances that can interfere with the assay and give false signals. Therefore negative controls, i.e. non-transfected or non-activated cells, should always be included when analysing cell extracts in the C-type RT Activity kit. All samples should also be analysed in serial dilutions.

Prepare the samples by resuspending the cell pellet in 1 to 2 ml of the prepared *C-type Sample Dilution Buffer* (see step A-4a or B-2a for instructions on how to prepare the buffer depending on protocol). Incubate the sample in room temperature for 15 minutes before analysis. The samples are best stored in the form of cell pellets at -70°C. Suspended samples may be aliquoted and stored at -70°C, but this may affect the RT activity depending on RT isozyme. The effect of storing suspended samples should therefore always be analysed.

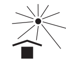
SERUM may contain substances, like RT blocking antibodies, that can inhibit the RT activity. It is therefore recommended to analyse the samples in four-fold serial dilutions, starting with a series that introduces 5 µl serum in the first well.

If particle associated RT activity is expected, the sensitivity of the assay may be enhanced by concentrating the particles through centrifugation (>20000 g/1 hour). Resuspend the pellet in the prepared *C-type Sample Dilution Buffer* (see step A-4a or B-2a for instructions on how to prepare the buffer depending on protocol) after centrifugation. Serum and suspended samples may be stored at -70°C. Avoid repeated freezing and thawing cycles.

Precautions

- For *in vitro* use only.
- For research purposes only.
- Observe normal precautions required for handling infectious material and laboratory reagents.
- Do not combine components from kits with different LOT numbers.

Symbols - used in instructions for use and kit labelling

	Manufacturer		Keep away from direct light		Article number
LOT	LOT number	-25°C / -14°C	Temperature limitation	Cat#	C-type RT Activity kit article number

PROTOCOL A: QUANTIFICATION OF RT ACTIVITY

A-1. COLLECT AND THAW COMPONENTS

a. Collect the following kit components and thaw the frozen ones at or below 37°C:

2 *Poly A Plate*
1 *C-type Sample Dilution Buffer*
2 *C-type Sample Dilution Components*
2 *C-type RT Reaction Components*
1 *C-type Reconstitution Buffer*
1 *MMuLV rRT Standard*
1 *C-type Plate Wash Buffer conc.*
2 Pieces of adhesive tape
2 Plastic lids for 96-well plates

A-2. PREPARE THE REACTION MIXTURE

a. Add 12 ml of *C-type Reconstitution Buffer* to each of the two *C-type RT Reaction Components*. Vortex for ten seconds.

b. Mix contents of both *C-type RT Reaction Components* with 12 ml of purified water in a 50-ml bottle, labelled “Reaction Mixture”. This makes up the Reaction mixture.

A-3. PREPARE THE POLY A PLATES

a. Take out both *Poly A Plates* from their pouches. Save one pouch for later use in step A-8.

b. Add 150 µl of the Reaction mixture, prepared in step A-2, to each well of both *Poly A Plates*. Put a lid on each *Poly A Plate*.

c. Incubate the plates for 20 to 60 minutes at 33°C. Perform steps A-4 and A-5 during this time.

A-4. PREPARE MMULV rRT STANDARD DILUTION SERIES

See page 2 for information on how to use the kit for other RT isozymes.

a. Add 5 ml of *C-type Sample Dilution Buffer* to each of the two *C-type Sample Dilution Components* and vortex for ten seconds. Transfer the dissolved contents of both *C-type Sample Dilution Components* back to the *C-type Sample Dilution Buffer*. Vortex for five seconds.

b. Add 2.1 ml of the prepared *C-type Sample Dilution Buffer* to the *MMuLV rRT Standard*. Vortex for ten seconds.

c. Take out twelve test tubes. Add 450 µl of *MMuLV rRT Standard* to the first test tube. Add 250 µl of *C-type Sample Dilution Buffer* to the following eleven test tubes.

d. Make a dilution series by transferring 200 µl from the first test tube to the second, change pipette tip and mix five times. Transfer 200 µl from the second test tube to the third, change pipette tip and mix five times etc, all the way to the 12th test tube.

A-5. DILUTE THE SAMPLES

a. Add 160 µl of *C-type Sample Dilution Buffer* to all wells in columns 1 to 10 of a 96-well microtitre plate (not supplied).

b. Add 40 µl of the first sample to be analysed to well A1. Add 40 µl of the second sample to be analysed to well A2 and so on until the first ten samples has been added (wells A1 to A10).

c. Add 40 µl of the next ten samples to wells E1 to E10, in the same manner as for samples 1 to 10.

DILUTION OF SAMPLES IN WELLS A1 TO A10:

d. Transfer 40 µl from wells A1 to A10 to their corresponding B wells, change pipette tip and mix five times.

e. Transfer 40 µl from wells B1 to B10 to their corresponding C wells, change pipette tip and mix five times.

f. Transfer 40 µl from wells C1 to C10 to their corresponding D wells, change pipette tip and mix five times. Discard the pipette tips.

DILUTION OF SAMPLES IN WELLS E1 TO E10:

g. Transfer 40 µl from wells E1 to E10 to their corresponding F wells, change pipette tip and mix five times.

h. Transfer 40 µl from wells F1 to F10 to their corresponding G wells, change pipette tip and mix five times.

i. Transfer 40 µl from wells G1 to G10 to their corresponding H wells, change pipette tip and mix five times.

A-6. ADD SAMPLES, BUFFER BLANKS AND MMULV rRT STANDARD TO POLY A PLATES

See figure 2 on page 10 for an illustration of the set-up of the *Poly A Plates*.

a. Collect the *Poly A Plates* from the incubator.

b. Transfer 50 µl of each sample dilution in the 96-well microtitre plate to the corresponding wells in columns 1 to 10 in both *Poly A Plates* (A1 to A1, B1 to B1 etc).

c. Add 50 µl of *C-type Sample Dilution Buffer* to wells E12 to H12 of both *Poly A Plates*.

d. Transfer 50 µl of the diluted MMuLV rRT Standard in the first test tube to well A11 of both *Poly A Plates*. Change pipette tip and transfer 50 µl of each dilution to the corresponding well in both plates (test tube 2 to well B11, test tube 3 to C11... test tube 8 to H11, test tube 9 to A12...test tube 12 to D12).

e. Seal both *Poly A Plates* with adhesive tape. Press the tape down firmly over each well to ensure proper sealing. Incubate the plates at 33°C, on an orbital shaker set on gentle agitation. Incubate the first plate for three hours and the second overnight (16 to 24 hours).

A-7. PREPARE PLATE WASH BUFFER

a. Slowly pour 75 ml of Triton X-100 into 1 litre of purified water containing a magnetic spinbar on continuous rotation. Make sure that the Triton X-100 dissolves completely, which takes approximately ten minutes.

b. Mix 25 ml of *C-type Plate Wash Buffer conc.* and the dissolved Triton X-100 in a 10-litre container. Adjust the volume to 10 litres with purified water and mix thoroughly.

c. Store the *C-type Plate Wash Buffer conc.* bottle at between 4 to 8°C for later use in step Q-4.

A-8. STOP THE RT REACTION ON THE FIRST POLY A PLATE BY WASHING

If you are uncertain of the performance of your plate washer, try the manual washing procedure described on page 11.

a. Collect the first *Poly A Plate* from the incubator after the three-hour incubation. Adjust the plate washer to pass 3 ml of wash fluid per well for each cycle.

b. Wash the plate two cycles. Turn the plate 180° between washes if possible. Remove residual fluid in the wells by tapping the plate upside down on absorbing cloth or paper. Leave the plate to dry upside-down for five to ten minutes.

c. Put the *Poly A Plate* back in its pouch and store it between -14 to -25°C.

d. Continue the assay the following day using the Product Quantification Protocol on page 8.

PROTOCOL B: SCREENING FOR RT ACTIVITY

B-1. COLLECT AND THAW COMPONENTS

a. Collect the following kit components and thaw the frozen ones at or below 37°C:

2 *Poly A Plate*
1 *C-type Sample Dilution Buffer*
2 *C-type Sample Dilution Components*
2 *C-type RT Reaction Components*
1 *C-type Reconstitution Buffer*
1 *MMuLV rRT Standard*
1 *C-type Plate Wash Buffer conc.*
2 Pieces of adhesive tape
2 Plastic lids for 96-well plates

B-2. PREPARE THE REACTION MIXTURE

a. Add 5 ml of *C-type Sample Dilution Buffer* to each of the two *C-type Sample Dilution Components* and vortex for ten seconds. Transfer the dissolved contents of both *C-type Sample Dilution Components* back to the *C-type Sample Dilution Buffer*. Vortex for five seconds.

b. Add 12 ml of *C-type Reconstitution Buffer* to each of the two *C-type RT Reaction Components*. Vortex for ten seconds.

c. Mix contents of both *C-type RT Reaction Components* and 12 ml *C-type Sample Dilution Buffer* and 12 ml of purified water in a 50-ml bottle, labelled “Reaction Mixture”. This makes up the Reaction mixture.

B-3. PREPARE THE POLY A PLATES

a. Take out both *Poly A Plates* from their pouches. Save one pouch for later use in step B-8.

b. Add 200 µl of the Reaction mixture, prepared in step B-2, to each well of both *Poly A Plates*. Put a lid on each *Poly A Plate*.

c. Incubate the plates for 20 to 60 minutes at 33°C. Perform steps B-4 and B-5 during this time.

B-4. PREPARE MMULV rRT STANDARD DILUTION SERIES

See page 2 for information on how to use the kit for other RT isozymes.

a. Add 420 µl of *C-type Sample Dilution Buffer*, prepared in step B-2a, to the *MMuLV rRT Standard*. Vortex for ten seconds.

b. Take out twelve test tubes. Add 350 µl of *MMuLV rRT Standard* to the first test tube. Add 250 µl of *C-type Sample Dilution Buffer* to the following eleven test tubes.

c. Make a dilution series by transferring 200 µl from the first test tube to the second, change pipette tip and mix five times. Transfer 200 µl from the second test tube to the third, change pipette tip and mix five times etc, all the way to the 12th test tube.

B-5. PREPARE THE SAMPLES

a. Collect the samples to be analysed (two 10-µl portions of each sample are required).

B-6. ADD SAMPLES, BUFFER BLANKS AND MMULV rRT STANDARD TO POLY A PLATES

See figure 3 on page 11 for an illustration of the set-up of the *Poly A Plates*.

a. Collect the *Poly A Plates* from the incubator.

b. Add 10 µl of sample 1 to well A1 of each *Poly A Plate*. Change pipette tip and add 10 µl of sample 2 to well B1 and so on until all 80 samples have been added to each plate. DO NOT use any wells in columns 11 or 12.

c. Add 10 µl of *C-type Sample Dilution Buffer* to wells E12 to H12 of both *Poly A Plates*.

d. Transfer 10 µl of the diluted *MMuLV rRT Standard* in the first test tube to well A11 of both *Poly A Plates*. Change pipette tip and transfer 10 µl of each dilution to the corresponding well in both plates (test tube 2 to well B11, test tube 3 to C11...test tube 8 to H11, test tube 9 to A12...test tube 12 to D12).

e. Seal both *Poly A Plates* with adhesive tape. Press the tape down firmly over each well to ensure proper sealing. Incubate the plates at 33°C, on an orbital shaker set on gentle agitation. Incubate the first plate for three hours and the second overnight (16 to 24 hours).

B-7. PREPARE PLATE WASH BUFFER

a. Slowly pour 75 ml of Triton X-100 into 1 litre of purified water containing a magnetic spinbar on continuous rotation. Make sure that the Triton X-100 dissolves completely, which takes approximately ten minutes.

b. Mix 25 ml of *C-type Plate Wash Buffer conc.* and the dissolved Triton X-100 in a 10-litre container. Adjust the volume to 10 litres with purified water and mix thoroughly.

c. Store the *C-type Plate Wash Buffer conc.* bottle at between 4 to 8°C for later use in step Q-4.

B-8. STOP THE RT REACTION ON THE FIRST POLY A PLATE BY WASHING

If you are uncertain of the performance of your plate washer, try the manual washing procedure described on page 11.

a. Collect the first *Poly A Plate* from the incubator after the three-hour incubation. Adjust the plate washer to pass 3 ml of wash fluid per well for each cycle.

b. Wash the plate two cycles. Turn the plate 180° between washes if possible. Remove residual fluid in the wells by tapping the plate upside down on absorbing cloth or paper. Leave the plate to dry upside-down for five to ten minutes.

c. Put the *Poly A Plate* back in its pouch and store it between -14 to -25°C.

d. Continue the assay the following day using the Product Quantification Protocol on page 8.

PRODUCT QUANTIFICATION PROTOCOL FOR PROTOCOLS A AND B

Q-1. COLLECT AND THAW COMPONENTS

a. Collect the following kit components and thaw the frozen ones at or below 37°C:

1 *C-type Plate Wash Buffer conc.*
2 *C-type RT Product Tracer*
1 *Substrate Tablet*
1 *Substrate Buffer*
2 Pieces of adhesive tape
2 Plastic lids for 96-well plates

Q-2. PREPARE TRACER

a. Prepare about 30 ml of purified water containing 1% Triton X-100.

b. Add 12 ml of the 1% Triton X-100 solution to each *C-type RT Product Tracer* and vortex for ten seconds. Pool contents of both *C-type RT Product Tracer* into a 50-ml bottle, labelled “Tracer”.

Q-3. PREPARE SUBSTRATE BUFFER

a. Add the *Substrate Tablet* to the thawed *Substrate Buffer*. Store it in the **dark** until use.

Q-4. PREPARE PLATE WASH BUFFER

a. Slowly pour 75 ml of Triton X-100 into 1 litre of purified water containing a magnetic spinbar on continuous rotation. Make sure that the Triton X-100 dissolves completely, which takes approximately ten minutes.

b. Mix 25 ml of *C-type Plate Wash Buffer conc.* and the dissolved Triton X-100 in a 10-litre bucket. Adjust the volume to 10 litres with purified water and mix thoroughly.

Q-5. COLLECT POLY A PLATE FROM FREEZER

a. Collect the first *Poly A Plate* from the freezer. Please note that this plate should not be washed in step Q-6.

Q-6. STOP THE RT REACTION BY WASHING

If you are uncertain of the performance of your plate washer try, the manual washing procedure described on page 11.

a. Collect the second *Poly A Plate* from the incubator. Adjust the plate washer to pass 3 ml of wash fluid per well for each cycle.

b. Wash the plate two cycles. Turn the plate 180° between washes if possible. Remove residual fluid in the wells by tapping the plate upside down on absorbing cloth or paper. Leave the plate to dry upside-down for five to ten minutes.

Q-7. ADD TRACER TO POLY A PLATE

a. Vortex the Tracer bottle for five seconds. Transfer 100 µl from the Tracer bottle to each well of both *Poly A Plates*, without touching the bottom of the wells.

b. Seal the plates with adhesive tape. Press the tape down firmly over each well to ensure proper sealing. Incubate for 90 minutes at 33°C, on an orbital shaker set on gentle agitation.

Q-8. REMOVE EXCESS TRACER BY WASHING

If you are uncertain of the performance of your plate washer, try the manual washing procedure described on page 11.

a. Collect the *Poly A Plates* from the incubator after the 90-minute incubation. Adjust the plate washer to pass 3 ml of wash fluid per well for each cycle.

b. Wash the plates four cycles. Turn the plate 180° between washes if possible. Remove residual fluid in the wells by tapping the plate upside down on absorbing cloth or paper. Leave the plate to dry upside-down for five to ten minutes.

Q-9. ADD SUBSTRATE BUFFER TO RT REACTION PLATE

a. Vortex the *Substrate Buffer*, prepared in step Q-3, for five seconds and add 125 µl to each well of both *Poly A Plates*, without touching the bottom of the wells. Remove bubbles, if any, in the wells with a clean pipette tip for each well. Cover the plates with plastic lids and incubate in the **dark** at room temperature.

Q-10. READ THE POLY A PLATES

a. Read the plates at A_{405} 30 minutes after addition of the substrate. Read the plates a second time after two hours and a third time the following day (16 to 24 hours after addition of substrate). Incubate the plates in the **dark** at room temperature between the readings.

DATA PROCESSING

The manual calculations described below can be programmed for automatic execution in e.g. an Microsoft Excel® spreadsheet.

PROTOCOL A: QUANTIFICATION OF RT ACTIVITY

Please note that the C-type RT Activity kit only gives a linear relationship between A_{405} and RT activity if the readings are within the linear range of the plate reader used.

When calculating the results, data from each reading time should be processed separately, i. e. values for standard curve and samples should be from the same reading time.

a. Plot the A_{405} of each MMuLV rRT Standard dilution (wells A11 to D12) against the concentration of MMuLV rRT present. The MMuLV rRT values are found on the Standard sheet included in the kit. Calculate the best regression line utilising the mean A_{405} of the buffer blanks (wells E12 to H12) as y-axis intercept.

b. Determine the RT activity of each well with the aid of the regression line. Only determine RT activity for wells giving an A_{405} within the linear range of the reader and having signals greater than two times the mean value of the buffer blanks.

c. Compare the RT activity values for long and short RT assay incubation for each sample using the information below:

- If the RT values are similar - calculate the mean value for the sample.
- If a higher value is obtained for the short RT reaction time - use this value. The lower value obtained with the long RT reaction time is normally due to RT product degradation.
- If a significant value is obtained for the long RT reaction time only - use this value.

d. Calculate the RT activity for undiluted samples by compensating for the dilution used in the assay.

e. Calculate the mean RT activity of the sample. Should significantly lower values be obtained for higher sample concentrations, this could be due to disturbing factors in the sample. Values obtained from these dilutions should be excluded from the calculation.

FIGURE 2: SET-UP OF THE POLY A PLATES FOR PROTOCOL A

		1	2	3	4	5	6	7	8	9	10	11	12
Conc. 1	A	1	2	3	4	5	6	7	8	9	10	S1	S9
Conc. 2	B	1	2	3	4	5	6	7	8	9	10	S2	S10
Conc. 3	C	1	2	3	4	5	6	7	8	9	10	S3	S11
Conc. 4	D	1	2	3	4	5	6	7	8	9	10	S4	S12
Conc. 1	E	11	12	13	14	15	16	17	18	19	20	S5	Bb
Conc. 2	F	11	12	13	14	15	16	17	18	19	20	S6	Bb
Conc. 3	G	11	12	13	14	15	16	17	18	19	20	S7	Bb
Conc. 4	H	11	12	13	14	15	16	17	18	19	20	S8	Bb

(1) = Sample number (Bb) = Buffer blank
(S1) = MMuLV rRT Standard

PROTOCOL B: SCREENING FOR RT ACTIVITY

Please note that the C-type RT Activity kit only gives a linear relationship between A_{405} and RT activity if the readings are within the linear range of the plate reader used.

When calculating the results, data from each reading time should be processed separately, i. e. values for standard curve and samples should be from the same reading time.

a. Plot the A_{405} of each standard dilution (wells A11 to D12) against the concentration of MMuLV rRT present. The MMuLV rRT values are found on the Standard sheet included in the kit. Calculate the best regression line utilising the mean A_{405} of the buffer blanks (wells E12 to H12) as y-axis intercept.

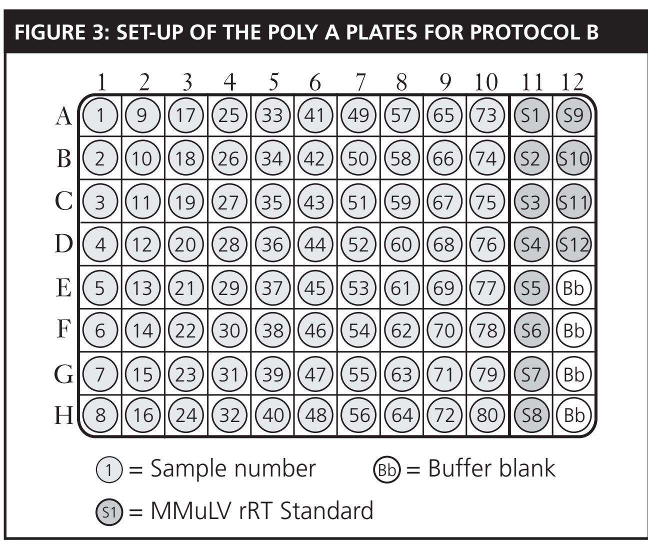
b. Determine the RT activity of each well with the aid of the regression line. Only determine RT activity for wells giving an A_{405} within the linear range of the reader and having signals greater than two times the mean value of the buffer blanks.

c. Compare the RT activity values for long and short RT assay incubation for each sample using the information below:

- If the RT values are similar - calculate the mean value for the sample.
- If a higher value is obtained for the short RT reaction time - use this value. The lower value obtained with the long RT reaction time is normally due to RT product degradation.
- If a significant value is obtained for the long RT reaction time only - use this value.

d. For samples where the first reading gives an A_{405} above the linear measuring range of the plate reader, the RT activity is above the highest MMuLV rRT activity in the standard curve. If an accurate value for such a sample is desired, the sample should be reanalysed using several dilutions (see Protocol A).

FIGURE 3: SET-UP OF THE POLY A PLATES FOR PROTOCOL B



PROTOCOL FOR MANUAL WASHING OF PLATES

Automatic plate washers can sometimes give too high A_{405} values in the buffer blanks and/or negative samples due to inadequate washing. If you are uncertain of the performance of your plate washer, try the following manual washing procedure. It can be used as a standard for calibration and control of automatic washing procedures.

a. Use four buckets of approximately 3 litres for the washing procedure. Prepare the Plate wash buffer as described in previous protocols. Pour 1 litre of the Plate wash buffer into each bucket (up to two plates can be washed simultaneously with this amount of buffer).

b. Collect the *Poly A Plate/Poly A Plates* from the incubator. Remove the tape carefully, pulling it diagonally from one corner to the other. Secure the strips with a rubber band. Empty the plate into the waste sink.

c. Put the plate in the first bucket with the wells facing up. Pick up the plate and empty it over the bucket. Repeat this action 15 times before moving the plate to the second bucket. Repeat the procedure in the second bucket and move the plate to the third bucket and so on.

d. After washing in four buckets, tap the plate upside-down on dry absorbing paper. Leave to dry upside-down on the paper for five to ten minutes. Tap the plate again to make all bubbles in the wells disappear. Do not forget to take off the rubber band before proceeding to the next step.

e. Throw away the used Plate wash buffer and rinse out the buckets, first with tap water and then with purified water. DO NOT use the same buffer for the two different wash steps (after RT reaction incubation and after RT Product Tracer incubation, respectively).

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