

Lenti RT Activity Kit

INSTRUCTIONS FOR USE

For Research Purposes Only

Cat# 51010

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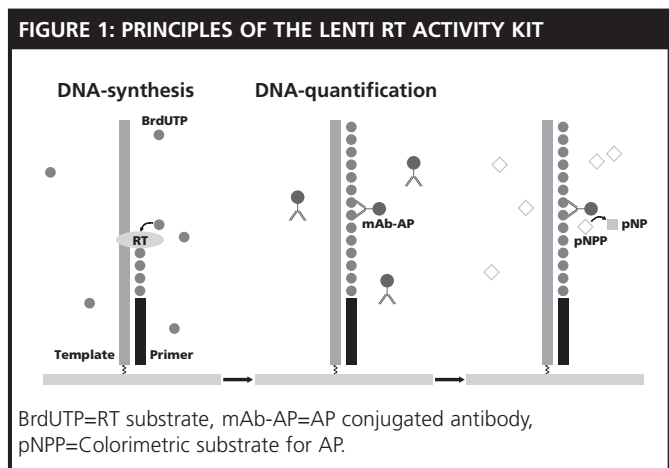
Intended Use

The Lenti RT Activity kit is intended for detection or quantification of the activity of the enzyme Reverse Transcriptase (RT) from lentivirus. The Lenti RT Activity Kit is not intended to be used as a screening test for HIV-1 infection in humans nor is it to be used as a diagnostic test to confirm the presence of HIV-1 infection in humans.

Principles of the Lenti RT Activity Kit

The Lenti 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.

A reaction mixture, containing primer and a nucleotide (BrdUTP), is added to the plate as well as the sample to be analysed. If the kit is used for determination of RT inhibitors, the substances tested should also be added. 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 Lenti RT Activity kit instructions for use describes three kit applications, quantification or screening for RT activity and determination of IC₅₀ values of RT inhibiting substances. The measuring range of the assay is 1 to 2000 pg RT/ml*.

PROTOCOL A: QUANTIFICATION OF RT ACTIVITY

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

TYPE OF SAMPLE: Cell culture supernatant

SAMPLE VOLUME: 40 μ l

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 HIV-1 rRT Standard set on each plate.

The measuring range of the assay corresponds to the activity of 0.05 to 100 pg recombinant HIV-1 RT per well. The lower detection limit corresponds to the presence of 5 pg/ml of active RT in undiluted cell supernatant, if the analysis is performed with the cell supernatant diluted 1:5, which is the highest concentration that can be used without risk of major disturbances. For comparison, the activity of 1 pg of the supplied HIV-1 rRT Standard is equal to 4 μ U RT activity¹. Other recombinant RT from lentiviruses may also be used instead of the included HIV-1 rRT.

PROTOCOL B: SCREENING FOR RT ACTIVITY

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

TYPE OF SAMPLE: Cell culture supernatant

SAMPLE VOLUME: 10 μ l + 10 μ l

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 HIV.

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 10, using the included HIV-1 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.

PROTOCOL C: DETERMINATION OF IC₅₀ VALUES OF RT ACTIVITY INHIBITING SUBSTANCES

MAXIMUM NUMBER OF INHIBITING SUBSTANCES THAT MAY BE ANALYSED WITH ONE KIT: 24

TYPE OF SUBSTANCES: Any RT inhibiting substance, except substances competing with other dNTPs than dTTP (assay based on prA/odT as template/primer)

SUBSTANCE VOLUME: 15 μ l

The Lenti RT Activity kit can be used to study RT inhibiting substances. To determine the IC₅₀ value, the substances to be analysed are serially diluted and a standardised amount of recombinant HIV-1 RT is mixed with each dilution. The ability of the RT to incorporate the substrate depends on the level of inhibition by the substance.

After correction for background signal, the measured residual RT activity for each substance dilution is calculated as a percentage of the measured RT activity in absence of inhibiting substance. The inhibitory effect of each substance is expressed as an IC₅₀ value, i.e. the substance concentration at which 50% of the RT activity is inhibited.

The kit can also be used for screening larger number of substances for inhibitory effects. In this case the substance is not serially diluted. A protocol for this applications is not provided.

Components

KIT COMPONENTS:

- 2 *Poly A Plate* (96 wells)
- 1 *Lenti Sample Dilution Buffer* (30 ml)
- 2 *Lenti RT Reaction Components* (lyophilised)
- 1 *Lenti Reconstitution Buffer* (25 ml)
- 1 *HIV-1 rRT Standard Lenti* (lyophilised)
- 1 *Plate Wash Buffer HS conc.* (50 ml)
- 2 *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 HIV-1 rRT Standard Sheet for Lenti RT Activity Kit

EQUIPMENT REQUIRED FOR PROTOCOL A ONLY

- 12 Test tubes (about 1 ml)
- 1 Standard 96-well microtitre plate with 300- μ l wells for titrations

DESCRIPTION OF COMPONENTS

- The *Poly A Plate* has pr(A) (polyriboadenosine) strands covalently bound to the wells.
- The *Lenti RT Reaction Components* contains lyophilised odT (oligodeoxythymidine) primer and BrdUTP (5-bromo-3-deoxyribouridine 5'-triphosphate).
- The *HIV-1 rRT Standard Lenti* contains lyophilised recombinant HIV-1 RT.
- The *RT Product Tracer* contains lyophilised monoclonal α -BrdUMP (anti bromo-deoxyribouridine mono-phosphate) 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.





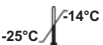
Sample Collection, Preparation and Storage

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.

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 number	 Temperature limitation	Cat# Lenti RT Activity kit article number

Step-by-Step Instructions

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 *Lenti Sample Dilution Buffer*
- 2 *Lenti RT Reaction Components*
- 1 *Lenti Reconstitution Buffer*
- 1 *HIV-1 rRT Standard Lenti*
- 1 *Plate Wash Buffer HS conc.*
- 2 Pieces of adhesive tape
- 2 Plastic lids for 96-well plates

A-2. PREPARE THE REACTION MIXTURE

- a. Add 12 ml of *Lenti Reconstitution Buffer* to each of the two *Lenti RT Reaction Components*. Vortex for ten seconds.
- b. Mix contents of both *Lenti 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 HIV-1 rRT STANDARD LENTI DILUTION SERIES

- a. Add 1.5 ml of *Lenti Sample Dilution Buffer* to the *HIV-1 rRT Standard Lenti*. Vortex for ten seconds.
- b. Add 250 µl of *Lenti Sample Dilution Buffer* to twelve test tubes.
- c. Add 100 µl of *HIV-1 rRT Standard Lenti* to the first test tube, change pipette tip and mix five times. 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 *Lenti 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 HIV-1 rRT STANDARD LENTI TO POLY A PLATES

See figure 2 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. 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 *Lenti Sample Dilution Buffer* to wells E12 to H12 of both *Poly A Plates*.
- d. Transfer 50 µl of the diluted HIV-1 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 *Plate Wash Buffer HS 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 *Plate Wash Buffer HS 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 13.

- 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 9.

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 *Lenti Sample Dilution Buffer*
- 2 *Lenti RT Reaction Components*
- 1 *Lenti Reconstitution Buffer*
- 1 *HIV-1 rRT Standard Lenti*
- 1 *Plate Wash Buffer HS conc.*
- 2 Pieces of adhesive tape
- 2 Plastic lids for 96-well plates

B-2. PREPARE THE REACTION MIXTURE

- a. Add 12 ml of *Lenti Reconstitution Buffer* to each of the two *Lenti RT Reaction Components*. Vortex for ten seconds.
- b. Mix contents of both *Lenti RT Reaction Components* with 12 ml *Lenti 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 HIV-1 rRT STANDARD LENTI DILUTION SERIES

- a. Add 300 µl of *Lenti Sample Dilution Buffer* to the *HIV-1 rRT Standard Lenti*. Vortex for ten seconds.
- b. Add 250 µl of *Lenti Sample Dilution Buffer* to twelve test tubes.
- c. Add 100 µl of *HIV-1 rRT Standard Lenti* to the first test tube, change pipette tip and mix five times. 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 is required).

B-6. ADD SAMPLES, BUFFER BLANKS AND HIV-1 rRT STANDARD LENTI TO POLY A PLATES

See figure 3 on page 12 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 *Lenti Sample Dilution Buffer* to wells E12 to H12 of both *Poly A Plates*.

- d. Transfer 10 µl of the diluted *HIV-1 rRT Standard Lenti* 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 *Plate Wash Buffer HS 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 *Plate Wash Buffer HS 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 13.

- 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 9.

PROTOCOL C: DETERMINATION OF IC₅₀ VALUE OF RT ACTIVITY INHIBITING SUBSTANCES

C-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 *Lenti Sample Dilution Buffer*
 - 1 *Lenti RT Reaction Components*
 - 1 *Lenti Reconstitution Buffer*
 - 1 *HIV-1 rRT Standard Lenti*
 - 2 Pieces of adhesive tape
 - 2 Plastic lids for 96-well plates

C-2. PREPARE THE SAMPLE DILUTION BUFFER

- a. Mix 30 ml of purified water and 30 ml *Lenti Sample Dilution Buffer* in a 100-ml bottle, labelled "SDB".

C-3. DILUTE THE SUBSTANCES

- a. Transfer 135 μl from the SDB bottle to each well of two 96-well microtitre plates (not supplied).
- b. Add 15 μl of the first substance to be analysed to well A1 of the first microtitre plate. Add 15 μl of the second substance to well A2 and so on until the first twelve substances have been added to wells A1 to A12 of the first microtitre plate.
- c. Add 15 μl of the 13th substance to be analysed to well A1 of the second microtitre plate. Add 15 μl of the 14th substance to well A2 and so on until substance 13 to 24 have been added to wells A1 to A12 of the second microtitre plate.
- d. In both microtitre plates, use a multi-channel pipette to mix the contents of wells A1 to A12 five times and transfer 15 μl to their corresponding B-wells. Change tips, mix five times and transfer 15 μl to their corresponding C-wells etc, all the way to row G.

C-4. PREPARE THE REACTION MIXTURE

- a. Add 2 ml of *Lenti Reconstitution Buffer* to the *Lenti RT Reaction Components*. Vortex for ten seconds.
- b. Transfer 200 μl from the *Lenti RT Reaction Components* back to the *Lenti Reconstitution Buffer*. Vortex for five seconds. This makes up the Reaction mixture.

C-5. PREPARE THE POLY A PLATES

- a. Add 100 μl of the Reaction Mixture, prepared in step C-4, to each well of both *Poly A Plates*.
- b. Transfer 50 μl from each well of the first microtitre plate to its corresponding well in the first *Poly A Plate*. Start with row H and work upwards to save pipette tips.
- c. Change tips and transfer 50 μl from each well of the second microtitre plate to its corresponding well in the second *Poly A Plate*.
- d. Put a lid on each *Poly A Plate*. Incubate the plates for 30 minutes at 33°C. Perform step C-6 during this time.

C-6. PREPARE HIV-1 rRT STANDARD LENTI

- a. Add 1.25 ml from the SDB bottle to the *HIV-1 rRT Standard Lenti*. Vortex for ten seconds.
- b. Mix 12 ml from the SDB bottle and 1 ml *HIV-1 rRT Standard Lenti* in a 50-ml bottle, labelled "RT STD".

C-7. ADD BUFFER BLANKS AND HIV-1 rRT STANDARD LENTI TO POLY A PLATES

See figure 5 on page 13 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 from the RT STD bottle to all wells EXCEPT H8 to H12 of both *Poly A Plates*.
- c. Transfer 50 μl from the SDB bottle to wells H8 to H12 of both *Poly A Plates*.
- d. Seal both *Poly A Plates* with adhesive tape. Press the tape down firmly over each well to ensure proper sealing. Incubate the plates for three hours at 33°C, on an orbital shaker set on gentle agitation.
- e. Continue the assay after the three-hour incubation, using the Product Quantification Protocol on page 9.

PRODUCT QUANTIFICATION PROTOCOL FOR PROTOCOLS A, B AND C

Q-1. COLLECT AND THAW COMPONENTS

- a. Collect the following kit components and thaw the frozen ones at or below 37°C:
 - 1 *Plate Wash Buffer HS conc.*
 - 2 *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 *RT Product Tracer* and vortex for ten seconds. Pool contents of both *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 *Plate Wash Buffer HS 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. FOR PROTOCOL A AND B ONLY: 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

For Protocol A and B - wash the second *Poly A Plate* only.

For Protocol C - wash both *Poly A Plates*.

If you are uncertain of the performance of your plate washer, try the manual washing procedure described on page 13.

- a. Collect the second *Poly A Plate* (Protocol A and B)/ both *Poly A Plates* (Protocol C) from the incubator. Adjust the plate washer to pass 3 ml of wash fluid per well for each cycle.
- b. Wash the plate/plates 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 13.

- 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

- 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.

PROTOCOL A & B

Q-10AB. READ THE POLY A PLATES

- 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.

PROTOCOL C

Q-10C. READ THE POLY A PLATES

- a. Read the plates at A_{405} 30 minutes after addition of the substrate.
- b. Repeat the reading of the plates until wells H1 to H7 give values between 10 to 80% of the maximum value within the linear range of the plate reader. 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.

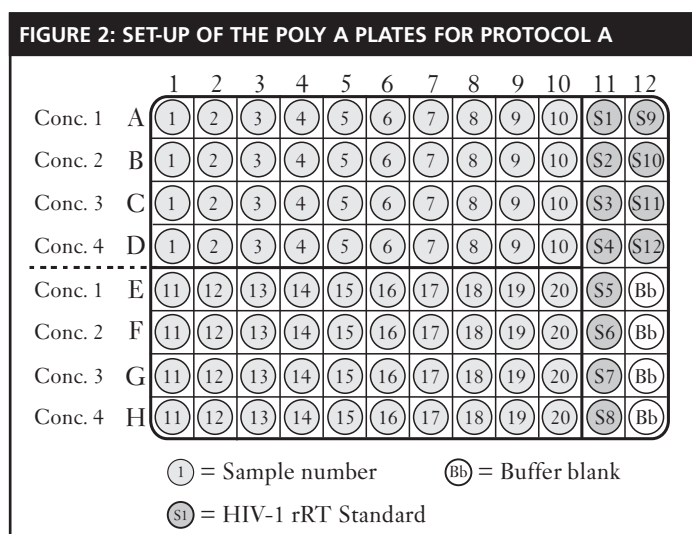
PROTOCOL A: QUANTIFICATION OF RT ACTIVITY

Please note that the Lenti 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 HIV-1 rRT Standard dilution (wells A11 to D12) against the concentration of HIV-1 rRT present. The HIV-1 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.



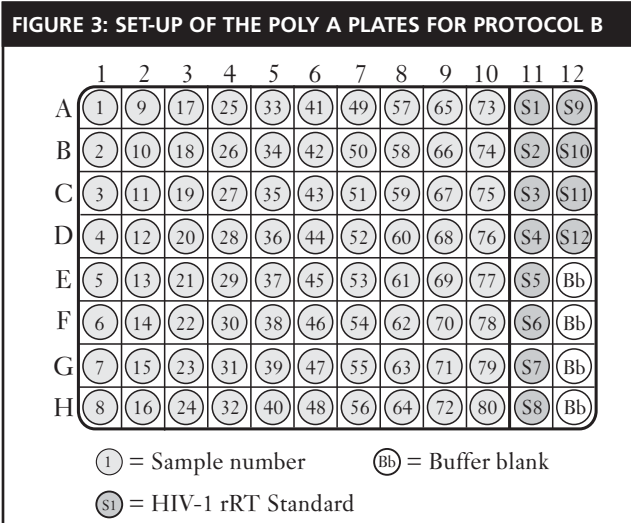
PROTOCOL B: SCREENING FOR RT ACTIVITY

Please note that the Lenti 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 HIV-1 rRT present. The HIV-1 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 HIV-1 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).



PROTOCOL C: DETERMINATION OF IC_{50} VALUES OF RT ACTIVITY INHIBITING SUBSTANCES

The IC_{50} value is the substance concentration that will inhibit the RT activity so that the remaining activity is 50% of the activity seen with no substance added.

For the IC_{50} calculations, use a reading time when wells H1 to H7 give approximately 10 to 80% of the maximum value within the linear measuring range of the plate reader. Follow the instructions below for each plate separately.

- a. Calculate the mean A_{405} value of the buffer blanks (wells H8 to H12).
- b. Subtract the mean A_{405} of the buffer blanks from the A_{405} value of all other wells.
- c. Calculate the mean A_{405} of the HIV-1 rRT Standard (wells H1 to H7).
- d. Express the A_{405} for wells containing substance dilutions as a percentage of the mean A_{405} value of the HIV-1 rRT Standard.
- e. Plot the percentage of residual RT activity on the Y-axis against the concentrations of the substance dilutions for each of the tested substances plotted on a logarithmic scale on the X-axis (see figure 4).
- f. Determine the log substance concentration on the X-axis of the point in the inhibition profile corresponding to 50% residual RT activity on the Y-axis.
- g. Calculate the IC_{50} value from the logarithmic X-axis value.

When the IC_{50} value is close to the lowest substance concentration used in the assay, a slightly more complicated algorithm, the median effect equation², may also be used. This is still essentially a log drug concentration versus remaining activity plot that can be described with a linear equation and programmed in e.g. Microsoft Excel[®].

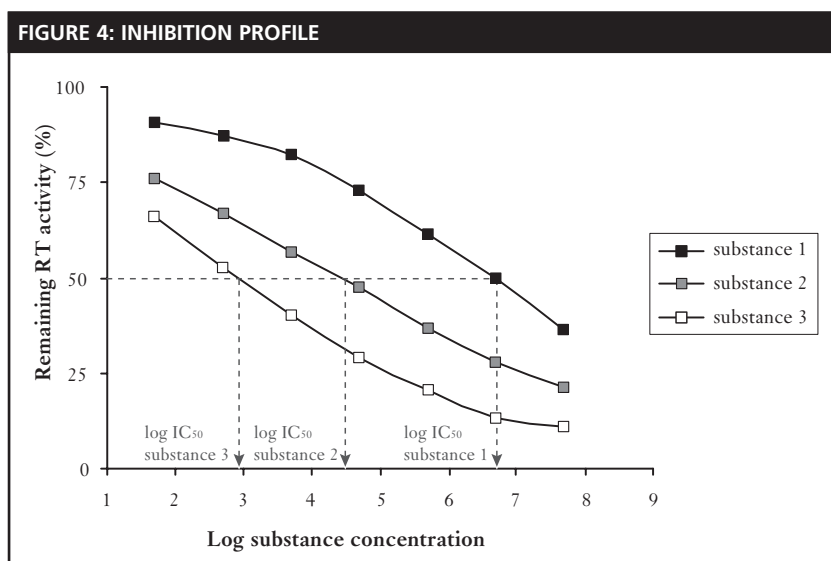
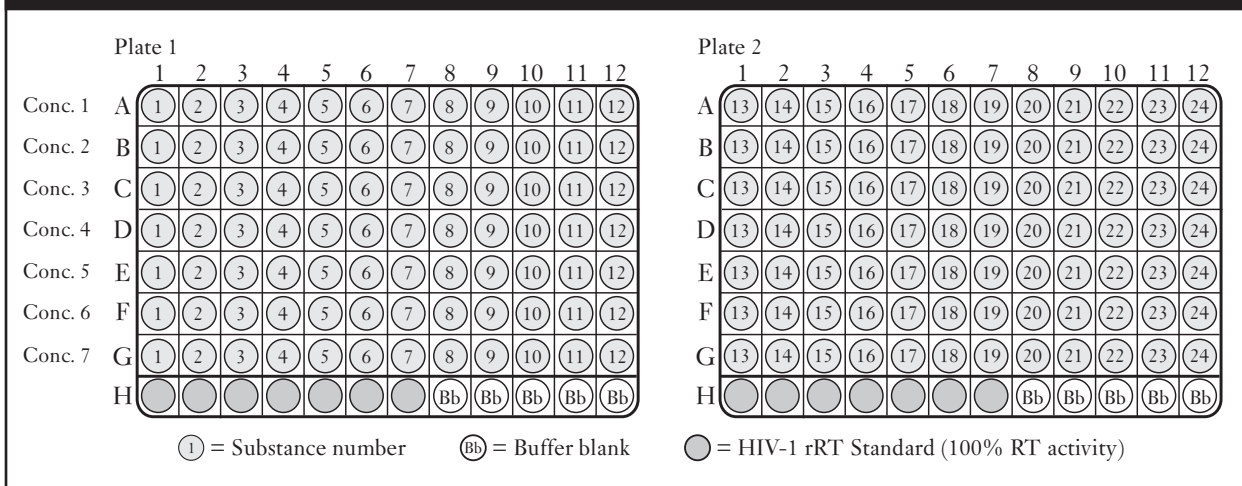


FIGURE 5: SET-UP OF THE POLY A PLATES FOR PROTOCOL C



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.

- 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).
- 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.
- 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.
- 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.
- 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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Cavidi AB

Uppsala Science Park
SE-751 83 Uppsala, Sweden

Tel: + 46 18 55 20 40

Fax: + 46 18 55 20 41

e-mail: info@cavidi.se

www.cavidi.com