

HS-Mg RT Activity Kit

INSTRUCTIONS FOR USE

For Research Purposes Only

Cat# 52020

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Intended Use

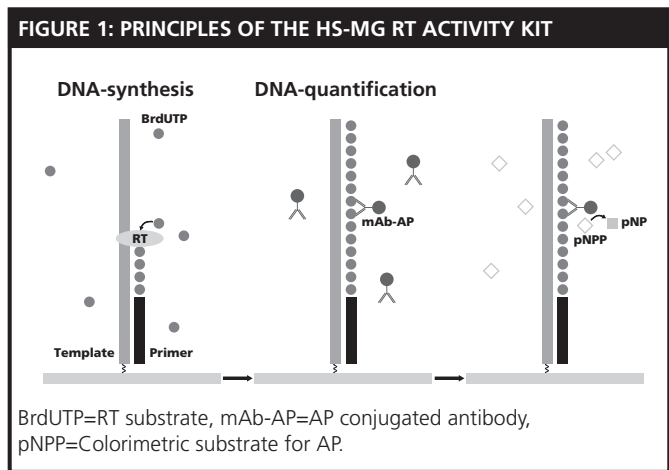
The HS-Mg RT Activity kit is intended for detection or quantification of the activity of Mg²⁺-dependent Reverse Transcriptase (RT) enzyme from retroviruses, e.g. Mouse Mammary Tumour Virus (MMTV) and HERV-K¹, a human endogenous retrovirus.

Principles of the HS-Mg RT Activity Kit

The HS-Mg 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 RT Reaction 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. 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 HS-Mg RT Activity kit instructions for use describes two kit applications, quantification and screening for RT activity. The measuring range of the assay is 0.5 to 500 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: 35 µl

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.

Although the assay is not optimised for HIV-1 RT, it functions well as a standard control. For comparison, the activity of 1 pg of the supplied HIV-1 rRT Standard is equal to 4 µU RT activity², when using the Cavid HS-Lenti RT Activity kit. Other recombinant Mg²⁺-dependent RT 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 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 MMTV.

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 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.

Components

KIT COMPONENTS

- 2 RT Reaction Plate (96 wells)
- 1 Base Buffer HS-Mg (58 ml)
- 2 Buffer Components (lyophilised)
- 2 RT Reaction Components HS (lyophilised)
- 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 HS-Mg 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 A₄₀₅ 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

EQUIPMENT REQUIRED FOR PROTOCOL A ONLY EQUIPMENT REQUIRED FOR PROTOCOL B ONLY

- 1 Test tube (about 5 ml)
- 1 Standard 96-well microtitre plate with 300- μ l wells for titrations

- 12 Test tubes (about 1 ml)

DESCRIPTION OF COMPONENTS

- The *RT Reaction Plate* has pr(A) (polyriboadenosine) strands covalently bound to the wells.
- The *RT Reaction Components HS* 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.
- 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.

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# HS-Mg 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 *RT Reaction Plate*
- 1 *Base Buffer HS-Mg*
- 1 *Buffer Components*
- 2 *RT Reaction Components HS*
- 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 BASE BUFFER HS-Mg

- a. Add 4 ml of *Base Buffer HS-Mg* to the *Buffer Components*. Vortex for ten seconds.
- b. Transfer 2 ml of the dissolved *Buffer Components* back to the *Base Buffer HS-Mg*. Vortex for five seconds.

A-3. PREPARE THE REACTION MIXTURE

- a. Add 12 ml of the *Base Buffer HS-Mg*, prepared in step A-2, to each of the two *RT Reaction Components HS*. Vortex for ten seconds. Save the remains of the *Base Buffer HS-Mg* for later use in step A-5 and A-6.
- b. Transfer the contents of both *RT Reaction Components HS* to a 50-ml bottle, labelled "Reaction Mixture". This makes up the Reaction mixture.

A-4. PREPARE THE RT REACTION PLATES

- a. Take out both *RT Reaction Plates* from their pouches. Save one pouch for later use in step A-10.
- b. Add 100 µl of the Reaction mixture, prepared in step A-3, to each well of both *RT Reaction Plates*. Put a lid on each *RT Reaction Plate*.
- c. Incubate the plates for 20 to 60 minutes at 33°C. Perform steps A-5 to A-7 during this time.

A-5. PREPARE THE SAMPLE PREPARATION PLATE

- a. Add 140 µl of the *Base Buffer HS-Mg*, prepared in step A-2, to all wells in columns 1 to 10 of a 96-well microtitre plate (not supplied). This is the sample preparation plate.
- b. Add 125 µl of *Base Buffer HS-Mg*, prepared in step A-2, to all wells in columns 11 to 12 of the sample preparation plate.

A-6. PREPARE HIV-1 rRT STANDARD LENTI DILUTION SERIES

- a. Add 4 ml of *Base Buffer HS-Mg*, prepared in step A-2, to the *HIV-1 rRT Standard Lenti*. Vortex for ten seconds.
- b. Mix 1 ml *Base Buffer HS-Mg* and 1 ml *HIV-1 rRT Standard Lenti* in a test tube.
- c. Add 100 µl of the diluted *HIV-1 rRT Standard Lenti* to well A11 of the sample preparation plate, change pipette tip and mix five times. Make a dilution series by transferring 100 µl from A11 to B11, change pipette tip and mix five times. Transfer 100 µl from B11 to C11, change pipette tip and mix five times etc...Transfer 100 µl from H11

to A12 , change pipette tip and mix five times etc. all the way to D12.

A-7. DILUTE THE SAMPLES

- a. Add 35 µl of the first sample to be analysed to well A1 of the sample preparation plate. Add 35 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).
- b. Add 35 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:

- c. Transfer 35 µl from wells A1 to A10 to their corresponding B wells, change pipette tip and mix five times.
- d. Transfer 35 µl from wells B1 to B10 to their corresponding C wells, change pipette tip and mix five times.
- e. Transfer 35 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:

- f. Transfer 35µl from wells E1 to E10 to their corresponding F wells, change pipette tip and mix five times.
- g. Transfer 35 µl from wells F1 to F10 to their corresponding G wells, change pipette tip and mix five times.
- h. Transfer 35 µl from wells G1 to G10 to their corresponding H wells, change pipette tip and mix five times.

A-8. ADD SAMPLES, BUFFER BLANKS AND HIV-1 RT STANDARD LENTI TO RT REACTION PLATES

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

- a. Collect the *RT Reaction Plates* from the incubator.
- b. Transfer 50 µl from each well of the sample preparation plate to the corresponding wells in the both *RT Reaction Plates* (A1 to A1, B1 to B1 etc).
- c. Seal both *RT Reaction 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-9. 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-10. STOP THE RT REACTION ON THE FIRST RT REACTION 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 *RT Reaction 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 *RT Reaction 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 *RT Reaction Plate*
 - 1 *Base Buffer HS-Mg*
 - 1 *Buffer Components*
 - 2 *RT Reaction Components HS*
 - 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 BASE BUFFER HS-Mg

- a. Add 4 ml of *Base Buffer HS-Mg* to the *Buffer Components*. Vortex for ten seconds.
- b. Transfer 2 ml of the dissolved *Buffer Components* back to the *Base Buffer HS-Mg*. Vortex for ten seconds.

B-3. PREPARE THE REACTION MIXTURE

- a. Add 17 ml of *Base Buffer HS-Mg*, prepared in step B-2, to each of the two *RT Reaction Components HS*. Vortex for ten seconds. Save the remains of the *Base Buffer HS-Mg* for later use in step B-5 and B-7.
- b. Transfer the contents of both *RT Reaction Components HS* to a 50-ml bottle, labelled "Reaction Mixture". This makes up the Reaction mixture.

B-4. PREPARE THE RT REACTION PLATES

- a. Take out both *RT Reaction Plates* from their pouches. Save one pouch for later use in step B-9.
- b. Add 140 µl of the Reaction mixture, prepared in step B-3, to each well of both *RT Reaction Plates*. Put a lid on each *RT Reaction Plate*.
- c. Incubate the plates for 20 to 60 minutes at 33°C. Perform steps B-5 and B-6 during this time.

B-5. PREPARE HIV-1 rRT STANDARD LENTI DILUTION SERIES

- a. Add 1.5 ml of *Base Buffer HS-Mg*, prepared in step B-2, to the *HIV-1 rRT Standard*

Lenti. Vortex for ten seconds.

- b. Add 250 µl of *Base Buffer HS-Mg* 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-6. PREPARE THE SAMPLES

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

B-7. ADD SAMPLES, BUFFER BLANKS AND HIV-1 rRT STANDARD LENTI TO RT REACTION PLATES

See figure 3 on page 10 for an illustration of the set-up of the *RT Reaction Plates*.

- a. Collect the *RT Reaction Plates* from the incubator.
- b. Add 10 µl of sample 1 to well A1 of each *RT Reaction 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 *Base Buffer HS-Mg* to wells E12 to H12 of both *RT Reaction Plates*.
- d. Transfer 10 µl of the diluted *HIV-1 rRT Standard Lenti* in the first test tube to well A11 of both *RT Reaction 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 *RT Reaction 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-8. 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-9. STOP THE RT REACTION ON THE FIRST RT REACTION 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 *RT Reaction 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 *RT Reaction 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 *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. COLLECT RT REACTION PLATE FROM FREEZER

- a. Collect the first *RT Reaction 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 *RT Reaction 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 RT REACTION PLATE

- a. Vortex the Tracer bottle for five seconds. Transfer 100 µl from the Tracer bottle to each well of both *RT Reaction 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 *RT Reaction 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 eight 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 100 µl to each well of both *RT Reaction 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 RT REACTION PLATES

- a. Read the plates at A_{405} 30 minutes after addition of the substrate. Repeat the reading at appropriate times, depending on the development of the colour signal. Readings after two hours and the following day is recommended. 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.

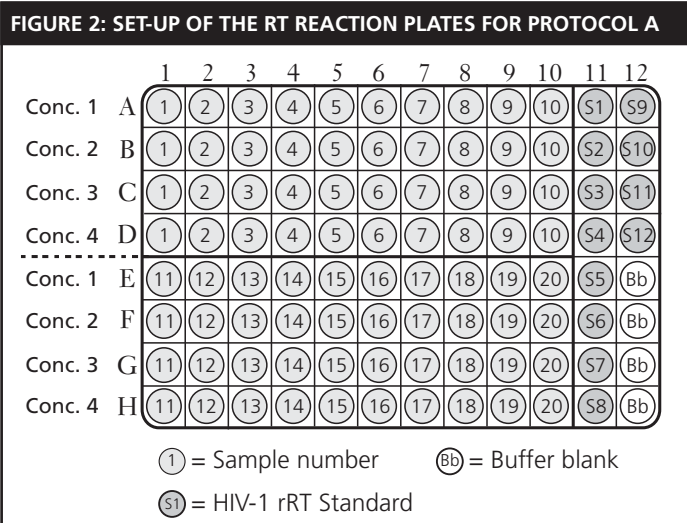
PROTOCOL A: QUANTIFICATION OF RT ACTIVITY

Please note that the HS-Mg 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.

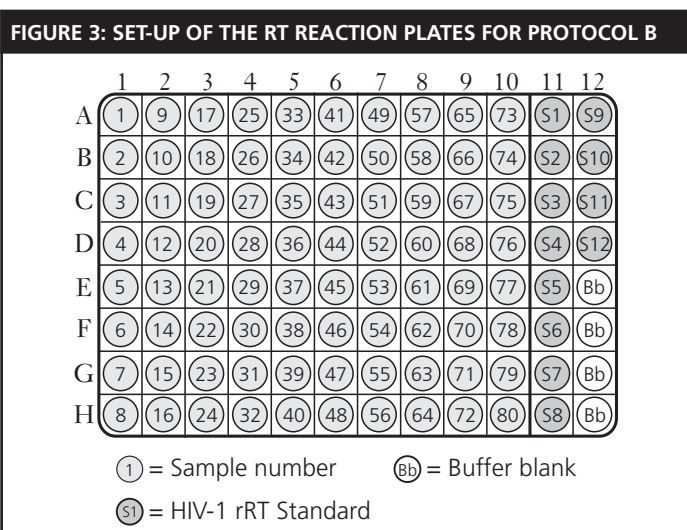


PROTOCOL B: SCREENING FOR RT ACTIVITY

Please note that the HS-Mg 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 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 *RT Reaction Plate/RT Reaction 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).

References

1. Lee YN, Bieniasz PD. Reconstitution of an infectious human endogenous retrovirus. *PLoS Pathog.* 2007 Jan;3(1):e10.
2. Ekstrand DHL, Awad RJ-K, Källander CFR and Gronowitz JS. A sensitive assay for the detection and quantification of RT activity, based on the use of carrier bound template and non-radioactive product detection, with special reference to HIV isolation. *Biotechnol Appl Biochem.* 1996 Apr;23 (Pt 2):95-105.

FURTHER READING

Lauren A, Thorstensson R, Fenyo EM. Comparative studies on mucosal and intravenous transmission of simian immunodeficiency virus (SIV_{sm}): the kinetics of evolution to neutralization resistance are related to progression rate of disease. *J Gen Virol.* 2006 Mar;87(Pt 3):595-606.

Manolov I, Raleva S, Genova P, Savov A, Froloshka L, Dundarova D, Argirova R. Antihuman Immunodeficiency Virus Type 1 (HIV-1) Activity of Rare Earth Metal Complexes of 4-Hydroxycoumarins in Cell Culture. *Bioinorg Chem Appl.* 2006; 2006: 71938.

Peters PJ, Sullivan WM, Duenas-Decamp MJ, Bhattacharya J, Ankghuambom C, Brown R, Luzuriaga K, Bell J, Simmonds P, Ball J, Clapham PR. Non-macrophage-tropic human immunodeficiency virus type 1 R5 envelopes predominate in blood, lymph nodes, and semen: implications for transmission and pathogenesis. *J Virol.* 2006 Jul;80(13):6324-32.

Aasa-Chapman MM, Holuigue S, Aubin K, Wong M, Jones NA, Cornforth D, Pellegrino P, Newton P, Williams I, Borrow P, McKnight A. Detection of antibody-dependent complement-mediated inactivation of both autologous and heterologous virus in primary human immunodeficiency virus type 1 infection. *J Virol.* 2005 Mar;79(5):2823-30.

Aquaro S, Svicher V, Ceccherini-Silberstein F, Cenci A, Marcuccilli F, Giannella S, Marcon L, Calio R, Balzarini J, Perno CF. Limited development and progression of resistance of HIV-1 to the nucleoside analogue reverse transcriptase inhibitor lamivudine in human primary macrophages. *J Antimicrob Chemother.* 2005 Jun;55(6):872-8.

Buscher K, Trefzer U, Hofmann M, Sterry W, Kurth R, Denner J. Expression of human endogenous retrovirus K in melanomas and melanoma cell lines. *Cancer Res.* 2005 May 15;65(10):4172-80.

Repits J, Oberg M, Esbjornsson J, Medstrand P, Karlsson A, Albert J, Fenyo EM, Jansson M. Selection of human immunodeficiency virus type 1 R5 variants with augmented replicative capacity and reduced sensitivity to entry inhibitors during severe immunodeficiency. *J Gen Virol.* 2005 Oct;86(Pt 10):2859-69.

Tuaille E, Gueudin M, Lemee V, Gueit I, Roques P, Corrigan GE, Plantier JC, Simon F, Braun J. Phenotypic Susceptibility to Nonnucleoside Inhibitors of Virion-Associated Reverse Transcriptase From Different HIV Types and Groups. *J Acquir Immune Defic Syndr.* 2004 Dec 15;37(5):1543-1549.

Azevedo-Pereira JM, Santos-Costa Q, Taveira N, Verissimo F, Moniz-Pereira J. Construction and characterization of CD4-independent infectious recombinant HIV-2 molecular clones. *Virus Res.* 2003 Nov;97(2):159-63.

Kempf W, Kadin ME, Dvorak AM, Lord CC, Burg G, Letvin NL, Koralnik IJ. Endogenous retroviral elements, but not exogenous retroviruses, are detected in CD30-positive lymphoproliferative disorders of the skin. *Carcinogenesis.* 2003 Feb;24(2):301-6.

Shao XW, Malmsten A, Lennerstrand J, Sönnerrborg A, Unge T, Gronowitz JS, Källander CFR: Use of HIV-1 reverse transcriptase recovered from human plasma for phenotypic drug susceptibility testing. *AIDS.* 2003 Jul 4;17(10):1463-71.

Corrigan GE, Al-Khalili L, Malmsten A, Thorstensson R, Fenyo E-M, Källander CFR and Gronowitz JS. Differences in reverse transcriptase activity versus p24 antigen detection in cell culture, when comparing a homogenous group of HIV-1 subtype B viruses with a heterogeneous group of divergent strains. *AIDS Res Hum Retroviruses.* 1998 Mar 1;14(4):347-52.

Awad RJ-K, Corrigan GE, Ekstrand DHL, Thorstensson R, Källander CFR and Gronowitz JS. Measurement of levels of HIV-1 reverse transcriptase (RT) and RT activity blocking antibody in human serum by a new standardized colorimetric assay. *J Clin Microbiol.* 1997 May;35(5):1080-9.

Shao X, Ekstrand DHL, Bhikhabhai R, Källander CFR and Gronowitz JS. A non-radioactive microtitre plate reverse transcriptase (RT) assay, based on immobilised template, for screening of RT inhibitors and evaluation of their mode of action. *Antivir. Chem. Chemother.* 1997;8:149-159.

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