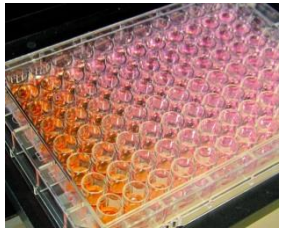
Instructions for use

ROTITEST®Vital

ready-to-use, sterile
Colorimetric test solution for evaluation of cell proliferation and -viability of adherent and suspension cells

A. Introduction

Rapid and simple test system for non-radioactive quantitation of proliferating cells and for cytotoxicity assays.
ROTITEST®Vital is based on the highly water-soluble tetrazolium salt WST-8[1], which is being dehydrogenased in living cells. Via the electron mediator 1-methoxy-PMS, reduction of NAD/NADP by the NAD dehydrogenase results in formation of an water-soluble orange formazan dye, which is subsequently released into the surrounding medium and may be measured photometrically (450 nm). Results are of highly stringent correlation with [^{3}H]-thymidine incorporation assays.[2]
Also suitable for culture media containing phenol red.



5 ml is sufficient for 500 measurements.

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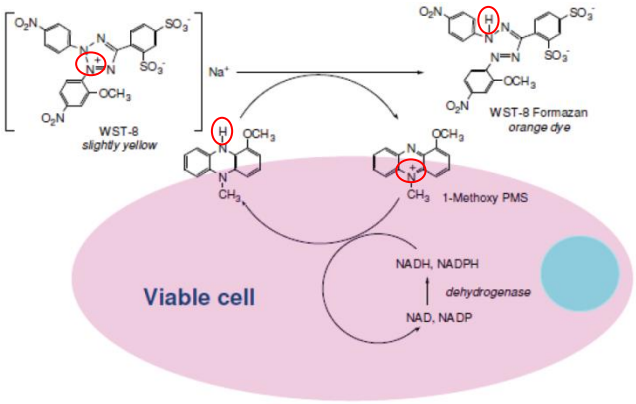


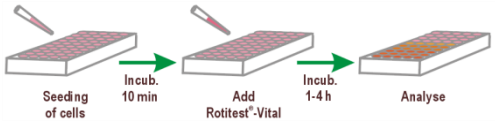
B. Storage and Usage

For frequent use, store at +4 °C protected from light. For long-term storage with infrequent use, store at -20°C. Repeated thawing and freezing causes increasing background and has to be avoided!

C. Application

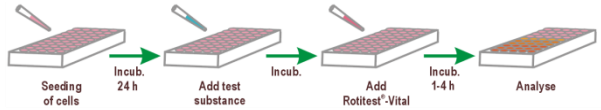
C.1 Cell Number Determination

1. Seed adherent cells or dispense cell suspension in a 96well plate (100 µl/well). Pre-incubate plate for 10 mins. (or longer, if required) in a humidified incubator (e.g., at 37 °C, 5 % CO_2).
2. Add 10 µl of ROTITEST®Vital solution to each well. Avoid formation of bubbles!
3. Incubate plate for 1-4 hours as in pre-incubation (1).
4. Measure absorbance at 450 nm using a microplate reader.



C.2 Cell Proliferation and Cytotoxicity Assay

1. Seed adherent cells or dispense cell suspension of 5x10^4 cells/ml in a 96-well plate (100 µl/well). Pre-incubate for 24 hours in a humidified incubator (e.g., at 37°C, 5 % CO_2).
2. Add test substances (10 µl in maximum).
3. Incubate plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.
4. Add 10 µl of ROTITEST®Vital solution to each well. Avoid formation of bubbles!
5. Incubate plate for 1-4 hours as in pre-incubation (1).
6. Measure absorbance at 450 nm using a microplate reader.



D. Tipps and Tricks, Important Notes:

1. Be careful not to introduce bubbles to the wells, since they interfere with measurement.
2. Specific test parameters like incubation time and cell number vary by the type of cells and may have to be optimised. Leukocyte derived cells, for instance, give weak staining, thus long incubation time (up to 4 hours) or a large number of cells (10^5 cells/well) may be necessary.
3. Adherent cells should be plated and grown to sub-confluency for cell proliferation and cytotoxicity assays.
4. Cell numbers: For adherent cells, use at least 1.000 cells/well. For leukocytes, use at least 2.500 cells/well) because of low sensitivity. Recommended maximum number of cells per 96well is 25.000.
5. In case bigger well plates shall be used, adjust the volume of medium, of ROTITEST®Vital solution, and number of cells accordingly by multiplying with the following factors: 48well - factor 3.1 / 24well - factor 6 / 12well - factor 12 / 6well - factor 30.
6. Plates may be conserved for later measurement. In this case add 10 µl of 1 % SDS or 0.1 M HCl to each well after the appropriate incubation time. Then cover the plate and store it at room temperature in the dark. Absorbance is kept constant for 24 hours.
7. Since the ROTITEST®Vital assay is based on the dehydrogenase activity detection in viable cells, conditions or chemicals that affect dehydrogenase activity may cause discrepancy between the actual viable cell number and the cell number determined.
8. WST-8 may react with reducing agents to generate WST-8 formazan. In case reducing agents are used in cytotoxicity- or cell proliferation assays, be sure to check background O.D.
9. ROTITEST®Vital solution is delivered sterile. If, however, sterilization has to be performed again, use membrane filtration.
10. Colour formation with ROTITEST®Vital solution is no end point reaction. Staining deepens even after

measurement. In case the signal has to be kept constant, add 10 µl of 1 % SDS or 0.1 M HCl to each well after the appropriate incubation time.

11. ROTITEST®Vital is compatible with other reagents for cell proliferation assays such as neutral red or crystal violet, which may be used subsequently.
12. In case of high turbidity in cell suspension, additionally measure $O.D._{600}$ and subtract values from $O.D._{450}$.
13. Since WST-8 and its formazan dye are highly water-soluble, ROTITEST®Vital cannot be utilized for cell staining purpose.
14. Phenol red in medium does not affect the assay. But make sure to use the same phenol red containing medium for controls.
15. Toxicity of ROTITEST®Vital is very low. The same cells may be used subsequently for other assays such as DNA fluorometric assay.
16. In case no filter with 450 nm is available, one can use filters with the absorbance between 430 and 490 nm. 450 nm filters give the best sensitivity, however.

E. Cells and Cell Lines

The following cells and cell lines have successfully been used with WST-8:

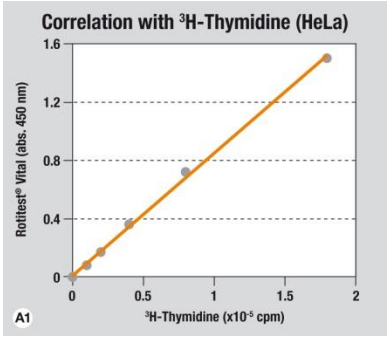
Cell lines: 10T1/2, 16HBE-T, 2008/C13*5.25, 293T, 3T3-L1, 3Y1, 786-0, 8505c, A2780, A2780cis, A431, A431/K5, A549, A6, ACHN, AGS, Alexander, ALVA-101, ALVA-41, AMO1, APL-NB4, APRE19, AR42J, ARO, ARPE-19, ASC, AsPC-1, ASTC-a-1, ATN-1, B16F1, B16F10, Ba/F3, Balb3T3, BBMVEC, BE(2)-C, BEAS-2B, BEL7404, BGC823, BHP15-3, BHP18-21v, BHP7-13, BMM, BMMSC, BMSC, BPH-1, BT-474, BxPC-3, C2C12, C3, C33A, C6, C8161.9, C8166, C8166-45, CA1, CA46, Ca9-22, Caco-2, Caco-2 cel, Caki-1, Caki-2, Caov-3, CDC, CGC, CHO, CLL, CMECs, CNE2 and T-47Ds, COLO 205, Colo320DM, COR-L23/P, COS-7, CV-1, D283-MED, Daoy, Daudi, DDLS, Dermal stem, DLD-1, DT40, DU145, E14TG2a, EC, EC9706, ESC, ES-E14TG2a, F11, F98 , F98s, FRCC001, FRCC562, FRH0201, FRO, FRT, FRTL-5, G361, GCIY, GES-1, Gin-1, GSM06, H1299, H1650, H1703, H1975, H23, H3255, H441, H9c2, HaCaT, HAK-1A, hAMSCs, hASM, HB1.F3, HCC, HCC1937, HCC827, HCC827 GR5, HCC827 GR6, HCECs, HCL, HCT116, HCT-8, HEK293, HEK293/4.63, HEK293/5I, HEK293T, HeLa, Hep3B, HepG2, HEY, HEYC2, hGal9, HL60, hMSC, hMVEC, HO-1-N-1, hPASMC, HSC-2, HSC-6, HSC-7, HSC-T6, HT22, HT-29, HTOA, HuCCT1, Huh-7, HUVEC, HXO-RB44, ILT-Hod, IMR32, INS-1, IPEC, J774, JB6 Pþ, JEG-3, JF, JHU-O11, Jurkat, K562, Kasumi-1, KB, KB-3-1, KC12, KK1, KMS-11, KMS-12PE, KMS-34, KOB, KOPN-8, KTC-2, KW-634, KW-807, KW-814, KW-857, KYN-2, KYN-3, KYSE, KYSE1170, KYSE170, KYSE410, L929, LAN-1, LC319, LCL, LCSC-2, LK87, LLC-PK1, LMB-H226-CL, LNCaP, LoVo, LS141, M059J, M059K, M28, Mast, MC38, MC3T3-E1, MC9, MCF-7, MDA-MB-231, MDA-MB-435, MDCK, MEF, mESC, MeT5A, mGCs, MH134, MHCC97H, MHCC97L, MH-S, MiaPaCa-2, MIN6, MKN1, MKN28, MKN45, MKN7, MKN74, MN9, MOLT-4, MPNST, MSC, MSTO-211H, MT1, MT2, MT4, Nb2, NCI-H226, Neuro2a, NHBE, NHDF, NHEK, NHF, NIH3T3, NOK-SI, NRK/SEAPs, NRK-52E, NS/PC, NSC, NT2N, OCI-Ly7, OMT, OPM1cell, OS-RC-2, OVCAR-3, OVK18, P493-6, PAMC82-P3, PANC-1, PBL, PBMC, PC12, PC14PE6, PC3, PC6, PC9, PCN, PFSK, PPC-1, PrE, PSC, PSN-1, QBC939, R1, R28, Raji, Ramos, RA-SF, RAW 264, RAW 264.7, rBCEC, RC, RERF-LC-OK, RGC-5, RIN5F, RL95-2s, RP9, RPE, S1, S1-M1-80, SBC-3, SBC-5, SCC-15, SH-10TC, SH-SY5Y, SK-BR-3, SK-Hep-1 , SK-N-AS, SH-SY5Y, SK-N-BE, SK-NFI, SK-N-SH, SH-SY5Y-Luc, SH-EP, SMS-KCN, SK-N-AS, MCF-10A, MCF-7, SM43, SMC-PV, SMS-KAN, SNU638, SPC, ST1, ST-8814, SUIT-2, SupT1, SW1990, BxPC3, SW480, SW620, SW839, T24, T-47D, T98G, Tca8113, T-Cell, TE13, TE6, TEC, THP-1, TK6, TL-Omi, TL-Su, TMK1, TRPV-OE, TS MEKK3-, TUHR10-TKB, TUHR14-TKB, H28, H2452, U251MG, U2OS, U-87, U87MG, U937, VAMT-1, vCT, Vero, Vero-shNuc-5, Vero-shGFP, VMRC-RCW, VSMC, WERI-RB-1, WI38VA-13, WSU-CLL, Y-79

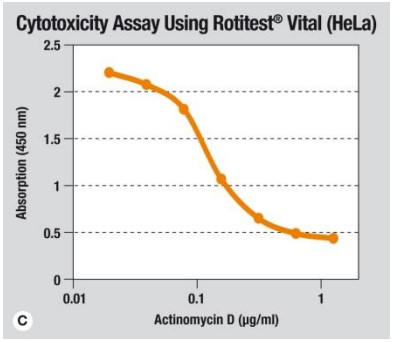
Primary cells: ATLs, brain, cardiac fibroblasts, conjunctival goblet, cortical neurons, HaCaT, Hippocampal neuron, medulla blastoma , MEMM, C57BL/6 mice

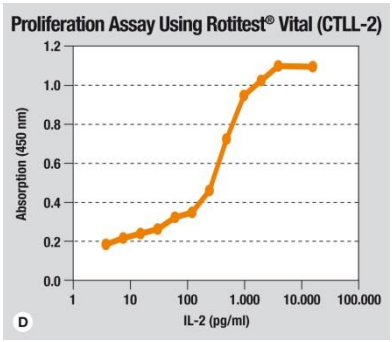
F. References

[1] [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-*2H*-tetrazolium, monosodium salt] Dojindo, Patent No. WO97/38985

[2] Tominaga M. *et al.* (1999) *Anal. Commun*. 36:47-50







ROTITEST®Vital	**0069.3**	**1ml**
	0069.1	**5 ml**
	0069.2	**4 x 5 ml**