



Tyrosinase RT-PCR ELISA

Detect melanoma cells in research samples by RT-PCR

Tyrosinase RT-PCR ELISA, a new photometric enzyme immunoassay, is specifically designed for the qualitative detection of tyrosinase mRNA after RT-PCR amplification in a highly sensitive and specific manner. The Tyrosinase RT-PCR ELISA combines the features of PCR with those of a standard ELISA, thereby offering a number of outstanding advantages over other systems.

The assay is based on the amplification of a tyrosinase-specific mRNA sequence by RT-PCR and subsequent detection of the amplicon by ELISA.

MelJu cell equivalent added to 5 ml of blood	Number of blood samples (different donors)	Tyrosinase positive samples (number)	Tyrosinase positive samples (%)
0	22	0	0
1	22	10	46
5	22	22	100

Table 1: Detection of tyrosinase mRNA in human blood.

5 ml aliquots of blood isolated from 22 healthy donors were lysed using RNA/DNA Stabilization Reagent for Blood/Bone Marrow. MelJu cell lysate equivalent to 1, 5, or 0 melanoma cells was added to the blood lysates of each donor and mRNA was prepared using the mRNA Isolation Kit for Blood/Bone Marrow. From each sample, mRNA corresponding to 1 ml of blood was used for tyrosinase detection using the Tyrosinase RT-PCR ELISA. Internal control: β -2-microglobulin.

Advantages of Tyrosinase RT-PCR ELISA

High sensitivity: The tyrosinase transcript is detectable in 40 fg of MelJu cell mRNA.

Easy handling of large numbers of samples: The ready-to-use reagents and the ELISA detection procedure allow fast and semi-automated processing of the samples.

Reliability: Combines with dedicated mRNA isolation kits to produce a system that facilitates convenient and controllable experimental designs.

Function-tested: The Tyrosinase RT-PCR ELISA is function-tested for the quality detection of melanoma cells spiked in blood that has been stabilized with the Blood/Bone Marrow Stabilization Reagent.

Sample material for the new kit can be purified mRNA (e.g., 100 ng mRNA) or total RNA isolated from human blood, or bone marrow, or tissue (e.g., lymph nodes). The kit is designed for 96 reactions; up to 46 samples can be tested depending on the number of samples analyzed per experiment. Samples can be analyzed in just 7 h.

Tyrosinase is specifically expressed by cells derived from the neuroectoderm, including melanocytes, melanoma cells, and peripheral glia. Since tyrosinase is specifically expressed in melanocytes, the enzyme can be viewed as a candidate for a cell line-specific marker for the detection of disseminated or metastatic melanoma cells by RT-PCR in sample materials that are normally tyrosinase negative (e.g., blood, bone marrow, or lymph nodes).

Procedure	Time
Part I: RT-PCR	approx. 3 h
Part II: nested PCR reaction	approx. 1.5 h
Part III: hybridization and photometric detection	approx. 2.5 h
Total hands-on time	approx. 1 h

Table 2: Assay time

This product is intended for medical research studies only.



Product	Cat. No.	Pack Size
NEW! Tyrosinase RT-PCR ELISA	2 013 762	96 reactions
MIA ELISA	1 976 826	96 reactions
Telomerase PCR ELISA	1 854 666	96 reactions
Cytokeratin-19 ELISA	1 865 536	96 reactions
p53 pan ELISA	1 828 789	96 reactions
mRNA Isolation Kit for Tissue	1 978 608	1 kit (25 isolations)
mRNA Isolation Kit for White Blood Cells	1 934 325	100 (30) isolations from 1.5 ml (5 ml) of sample material
mRNA Isolation Kit for Blood/Bone Marrow	1 934 333	100 (30) isolations from 1.5 ml (5 ml) of sample material
RNA/DNA Stabilization Reagent for Blood/Bone Marrow	1 934 317	for 50 ml of sample material
Aerosol-resistant Pipette Tips	1 667 068	960 tips (0.1–10 μ l)
Thin-walled PCR Tubes, (DNase and RNase-free)	1 667 041	1000 tubes (200 μ l)
	1 667 050	1000 tubes (500 μ l)

These products are accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased (i.e., an authorized thermal cycler). These products are sold under licensing arrangements with Roche Molecular Systems and The Perkin-Elmer Corporation.