

p53 pan ELISA

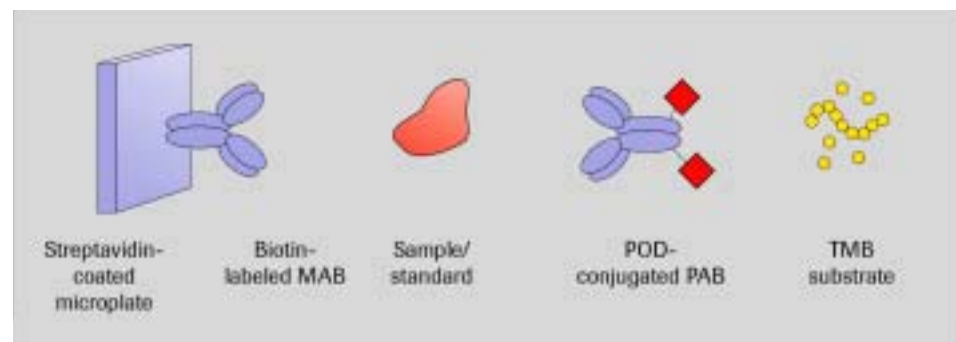
Cat. No. 11 828 789 001 96 tests

Type	One-step sandwich ELISA, colorimetric
Useful for	Quantitation of p53 protein, both wild-type and mutant forms
Samples	Tissue homogenates, cell lysates, serum, or plasma
Method	Lysis/homogenization of sample, followed by immunochemical determination of p53 in a microplate
Time	Approx. 2.5 h

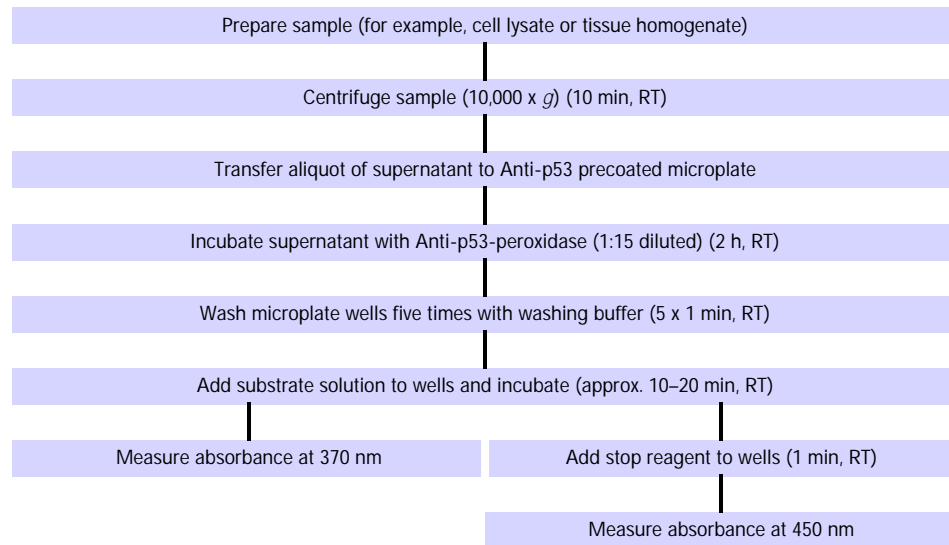
Significance of kit: This kit uses antibodies to quantitate the levels of human, mouse, or rat p53 in smear/plasma, in tumor tissue, or in tumor cell lines. Wild type p53 is a protein that suppresses malignant transformation by inducing apoptosis. Mutations of the p53 gene which increases its stability allow transformation (immortalization) of the cell and are quite commonly found in malignancies. The p53 protein seems also to be involved in cell death induced by cytotoxic drugs.

Test principle: The assay uses a one-step sandwich immunoassay (Figure 40) to detect wild-type and mutant p53. The procedure (Flow Chart 11) involves:

- 1 Preparing sample (*e.g.*, detergent lysis of cells, homogenization of tissue), followed by centrifugation.
- 2 Transferring an aliquot of the sample supernatant to streptavidin-coated well of a microplate (precoated with anti-p53-biotin).
- 3 Binding p53 in the supernatant with two antibodies, a biotin-labeled monoclonal anti-p53 (capture antibody), which is pre-bound to the streptavidin-coated plate, and a peroxidase-labeled polyclonal anti-p53 (detection antibody).
- 4 Washing the immobilized antibody-p53 complexes five times to remove sample components that are not immunoreactive.
- 5 Incubating sample with peroxidase substrate (tetramethylbenzidine, TMB).
- 6 Determining the amount of colored product (and thus, of immobilized antibody-p53 complexes) spectrophotometrically.



▲ Figure 40: How the p53 pan ELISA works.



▲ Flow Chart 11: Assay procedure, p53 pan ELISA.

Sensitivity: In 4 independent assays, the lower limit of detection for the assay was determined to be 9 pg/ml.

Specificity: The biotin-labeled capture antibody from mouse recognizes a conserved, pantropic, denaturation stable antigenic determinant of the p53 protein (human, mouse, rat). The peroxidase-labeled detection antibody is highly specific for wild-type and mutant p53 from different species.

Can be used to assay:

- Tissue homogenates
- Cell lysates
- Serum or plasma

Kit contents

1. Anti-human-p53 pan, polyclonal, peroxidase-labeled
2. Human p53 standards (six)
3. Incubation buffer/Sample diluent, ready-to-use
4. Washing buffer, 10 x concentrated
5. TMB substrate solution
6. TMB stop solution
7. Streptavidin-coated microplate, precoated with monoclonal Anti-p53 (biotin-labeled)
8. Adhesive plate cover foils