Enzo Life Sciences

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ImmunoSet® p53/MDM2 complex ELISA development set
Catalog # ADI-960-070
Reagents for 5 x 96-Well EIA Kits

This ImmunoSet contains the basic components for the development of a p53/MDM2 complex-specific immunometric enzyme immunoassay (EIA). Each kit contains sufficient reagents for five 96-well plates.

This kit has been validated for use with cell lysates. Additional sample types will require validation by the user.

Visit www.enzolifesciences.com for tips and frequently asked questions.

Introduction

The p53 tumor suppressor protein is a transcription factor that regulates a variety of biological functions including growth arrest, apoptosis, DNA repair, and differentiation[1,2]. MDM2 is the principal antagonist of p53, and evidence suggests *MDM2* gene amplification is found in about 7% of tumor tissues[3,4]. MDM2 acts as an E3 ubiquitin ligase, cooperating with p300/CBP in the ubiquitination and subsequent proteosomal degradation of p53 in the cytoplasm and nucleus. Various strategies for cancer therapy involve activating the apoptotic and growth suppressive functions of p53 by disrupting its association with MDM2[5].

References:

1. Levine, A.J. (1997) Cell **88**, 323-331.
2. Vousden, K.H. and Lu, X. (2002) Nat. Rev. Cancer **2**, 594-604.
3. Momand, J., *et al.* (1998) Nucleic Acids Res. **2**, 3453-3459.
4. Oliner, J.D., *et al.* (1992) Nature **358**, 80-83.
5. Chene, P. (2004) Mol. Cancer Res. **2**, 20-28.

Materials Provided

1. p53 Capture Antibody
   One vial containing 312.5 µg lyophilized p53 monoclonal antibody, Cat. #80-1893
2. p53 (human) Standard
   One vial containing 500 ng lyophilized recombinant p53 protein, Cat. #80-1892
3. MDM2 (human) Standard
   One vial containing 160 ng lyophilized recombinant MDM2, Cat. #80-1891
4. MDM2 Detection Antibody
   One vial containing 2.5 µg lyophilized biotinylated MDM2 polyclonal antibody, Cat. #80-1894
5. SA-HRP
   One vial containing 12.5 µg lyophilized SA-HRP, Cat. #80-1896

Materials Needed but not Supplied

1. RIPA Cell Lysis Buffer 2, Cat. #80-1284, or similar
2. 96-well high-binding polystyrene microtiter plates, Cat. #80-1930, or similar
3. Precision pipets
4. Microplate reader capable of reading at 450 nm
5. Phosphate buffered saline (PBS)†
6. Tween®-20*†
7. Bovine Serum Albumin (BSA)†
8. 3,3',5,5' tetramethylbenzidine (TMB) solution, Cat. #80-1805, or similar†
9. 1N hydrochloric acid, such as Stop Solution 2, Cat. #80-1804†

†ImmunoSet Buffer Pack, Cat. #ADI-950-003
*Tween is a registered trademark of ICL Americas

Buffer Formulations

1. Coating Buffer
   10 mM sodium phosphate, 15 mM NaCl, pH 7.4
2. Blocking Buffer
   10 mM sodium phosphate, 15 mM NaCl, 1.0% BSA, pH 7.4
3. Assay Buffer
   100 mM sodium phosphate, 150 mM NaCl, 1.0% BSA, 0.1% Tween-20, pH 7.4
4. Wash Buffer
   10 mM sodium phosphate, 15 mM NaCl, 0.1% Tween-20, pH 7.4

Plate Coating

1. Reconstitute p53 Capture Antibody with 250 µL deionized water for a 250x stock. Use immediately, or make aliquots and freeze at -20°C for up to 3 months. For prolonged storage, aliquot and freeze at -80°C. Avoid repeated freeze/thaw cycles.
2. Dilute the stock 1:250 in Coating Buffer. Immediately dispense into 96-well microtiter plates using 100 µL of the diluted capture antibody per well. Seal the plate and incubate overnight at room temperature.
3. Aspirate each well to remove coating solution. Immediately add 200 µL Blocking Buffer per well. Seal the plate and incubate for at least 1 hour.
4. Aspirate each well to remove blocking solution. Plates may be used immediately or dried and stored with desiccant at 4°C.

Reagent Preparation

1. Recombinant p53/MDM2 Complex

   Reconstitute the p53 and MDM2 standards by adding 250 µL Assay Buffer to each vial. Vortex, wait 5 minutes and vortex again. Combine contents and incubate for 1 hour at room temperature to make a 20x stock of p53/MDM2 complex. The final concentration of this stock is 1 µg/mL p53 and 0.32 µg/mL MDM2. Aliquot and store at -20°C for up to 3 months. For prolonged storage, freeze at -80°C. Avoid repeated freeze/thaw cycles.

   The recommended standard curve range is 50 ng/mL to 0.8 ng/mL p53 protein. Gently thaw an aliquot of 20x stock and dilute 20-fold with Assay Buffer, followed by 2-fold serial dilutions in Assay Buffer. Do not store diluted standard. Please see Note under Typical Data.

2. MDM2 Detection Antibody

   Reconstitute vial contents with 250 µL deionized water for a 250x stock. Store at 4°C for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles.

   Dilute the stock 1:250 in Assay Buffer for a working solution. Do not store diluted antibody.

3. SA-HRP

   Reconstitute vial contents with 250 µL deionized water for a 1000x stock. Store at 4°C for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles.

   Dilute the stock 1:1000 in Assay Buffer for a working solution. Do not store diluted conjugate.

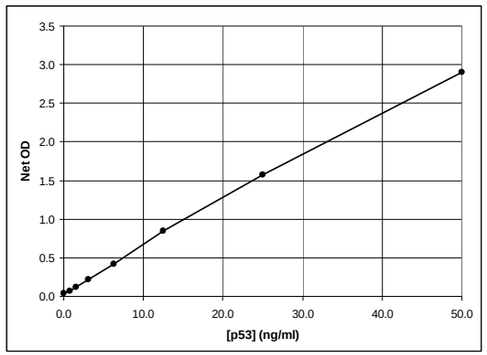
Assay Procedure

1. Pipet 100 µL of Assay Buffer into the control (0 ng/mL standard) wells.
2. Pipet 100 µL of standards and samples, prepared in Assay Buffer, to the bottom of the appropriate wells.
3. Seal the plate. Incubate on a plate shaker for 1 hour at room temperature.
4. Empty the contents of the wells and wash by adding 400 µL of Wash Buffer to every well. Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
5. Pipet 100 µL of the diluted detection antibody into each well, except the blank.
6. Seal the plate. Incubate on a plate shaker for 1 hour at room temperature.
7. Wash as above (Step 4).
8. Add 100 µL of the diluted conjugate to each well except the blank.
9. Seal the plate. Incubate on a plate shaker for 30 minutes at room temperature.
10. Wash as above (Step 4).
11. Pipet 100 µL of TMB solution into each well.
12. Seal the plate. Incubate on a plate shaker for 30 minutes at room temperature.
13. Pipet 100 µL 1N HCl into each well.
14. After blanking the plate reader against the substrate, read optical density at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Assay Performance

Typical Data

The results shown below are for illustration only and should not be used to interpret results from another assay.



Note: This assay is designed to provide a semi-quantitative determination of p53/MDM2 complex levels. These proteins are known to form a complex containing one p53 tetramer to one MDM2 monomer, and the recombinant standard mixture contains a 4:1 molar ratio of p53 to MDM2. However, the precise stoichiometry and stability of the resulting p53/MDM2 complex have not been determined. For analytical purposes, the standard concentration is reported relative to the amount of p53 present.

Sensitivity

The sensitivity, or limit of detection, of this assay is 0.35 ng/mL. It was determined by interpolation at 2 standard deviations above the mean signal at background, using data from 10 standard curves.

Specificity

This assay detects p53/MDM2 in cell lysates from human, mouse, and rat sources. It does not show significant cross-reactivity with recombinant human p21 or Bax proteins.

Dilutional Linearity

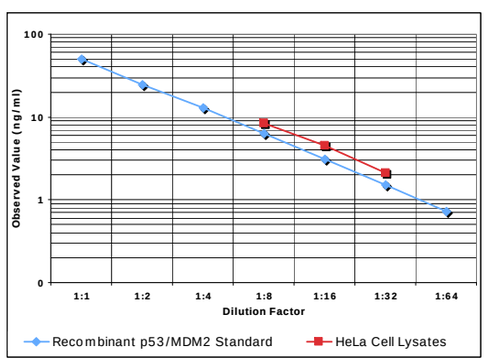
To determine possible interference from the sample matrix, cell lysates obtained using RIPA buffer were serially diluted into assay buffer. The levels of p53/MDM2 complex were measured in the assay, and the results were analyzed to determine the range over which a linear response was obtained. From these data, the minimum recommended dilution (MRD) is 1:8 for similar samples.

Dilution Factor	HeLa CL	3T3 CL	C6 CL
Neat	---	---	---
1:2	---	---	---
1:4	67%	62%	73%
1:8	101%	99%	113%
1:16	109%	102%	112%
1:32	100%	100%	100%

CL: Cell Lysate

Parallelism

Dose-response curves from cell lysates diluted into assay buffer (using the MRD) were compared to the recombinant p53/MDM2 standard. Parallelism indicates that the antibody-binding characteristics of the native and standard proteins are similar, allowing accurate determination of the analyte.



Calculation of Results

Several options are available for the calculation of the relative levels of p53/MDM2 complex in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve-fitting program. For accuracy, please ensure that sample values fall within the standard range. Please see Note under Typical Data.

Accessory Reagent List		
Reagent	Quantity	Cat. #
ImmunoSet® Buffer Pack	1 each of the following products: 80-1927, 80-1928, 80-1929, 80-1805, 80-1804	ADI-950-003
ImmunoSet® Plate Pack	5 96-well clear microtiter plates & 5 plate sealers	80-1930
PBS Concentrate	120 mL	80-1927
BSA Solution (10%)	50 mL	80-1928
Tween-20 Solution (10%)	30 mL	80-1929
RIPA Cell Lysis Buffer 2	100 mL	80-1284
TMB Substrate	50 mL	80-1805
1N HCl Stop Solution	50 mL	80-1804
SA-HRP	12.5 µg/vial	80-1896

Storage

Store all components at 4°C, except the standards. Standards must be stored frozen at -20°C or below. See pages 3-4 for storage of reconstituted material.

Tips & Troubleshooting

- ✓ If buffers other than those recommended are used in the assay, the end-user must determine the appropriate dilution and assay validation.
- ✓ Pipet the reagents to the sides of the wells to avoid possible contamination.
- ✓ Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.
- ✓ Insufficient washing or residual wash buffer in the wells may cause variation in assay results.
- ✓ Bring all reagents to room temperature for at least 30 minutes prior to opening.
- ✓ All standards, controls, and samples should be assayed in duplicate.

Limited Warranty

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