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ab154470 – p53 Immunocapture Kit

Instructions for Use

Abcam's p53 Immunocapture Kit is designed to immunocapture p53 from whole cell lysates

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's p53 Immunocapture kit is designed to immunocapture p53 from whole cell lysates. Traditional immunoprecipitation methods usually result in co-elution of the antibody heavy and light chains that may co-migrate with relevant bands, masking important results. The Abcam p53 immunocapture kit resolves this issue by immobilizing the p53 capture antibody onto protein G-agarose beads. The kit includes optimized buffers and reagents for sample preparation and p53 binding and recovery, which shorten the protocol and minimize handling and mixing.

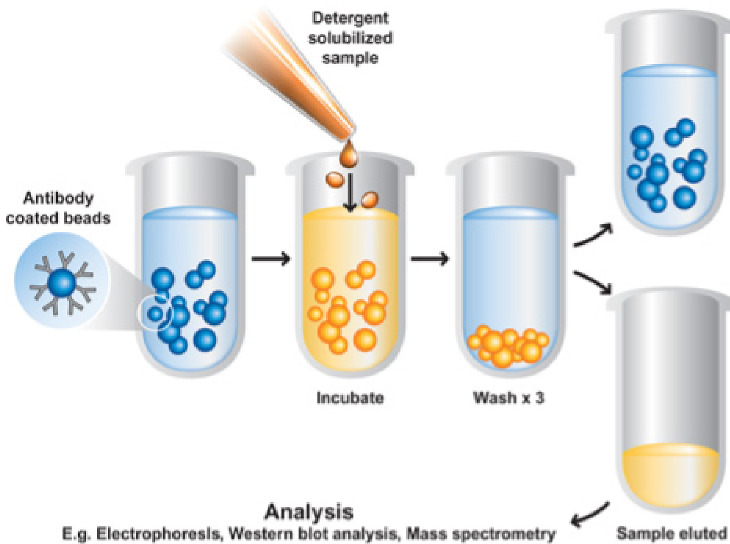
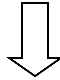


Figure 1. Schematic showing the various stages of the immunocapture assay.

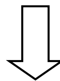
p53 acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. It is involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction by p53 seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. p53 is implicated in Notch signaling cross-over. p53 prevents CDK7 kinase activity when associated to CAK complex in response to DNA damage, thus stopping cell cycle progression.

2. ASSAY SUMMARY

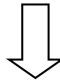
Solubilize samples in Extraction Buffer. Incubate on ice for 20 minutes



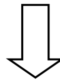
Centrifuge at 18,000 x g for 20 minutes at 4°C. Collect supernatant.



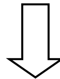
Incubate supernatant with diluted beads overnight at 4°C



Pellet beads by gentle centrifugation (2 minutes at 300 x g min) and wash the beads three times in Wash Buffer.



Elute p53 from the beads using 1X SDS Elution Buffer.



Analyze the eluted sample by protein electrophoresis or store the purified p53 at -80°C

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at +2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X Wash Buffer	10 mL	+2-8ºC
10X PBS	10 mL	+2-8ºC
10X Extraction Buffer	1 mL	+2-8ºC
Immunocapture p53 antibody coupled to agarose beads	200 µg	+2-8ºC
1X SDS Elution Buffer	1 mL	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Method for determining protein concentration (BCA assay recommended)
- Nanopure water
- Single channel pipettes
- Protease inhibitor cocktail (e.g. ab65621 from Abcam)
- Laboratory benchtop microcentrifμge
- Protein electrophoresis equipment
- Tube rotation equipment
- pH meter, weigh balance and other standard lab equipment

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **Sample extraction:** The provided lauryl maltoside extraction buffer is formulated to be non-denaturing to preserve protein-protein interactions useful for co-immunoprecipitation. However, other types of extraction conditions are compatible with this kit. Figure 1 shows the immunocapture beads are also compatible with RIPA extraction buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) and SDS extraction buffer (1% SDS in 1X PBS). Note that the SDS extracts must be diluted 20-fold prior to incubation with antibody loaded beads to avoid antibody denaturation.
- **Using p53 antibody coated agarose bead slurry:** Since the beads are a solid material they cannot be pipetted directly. Instead they are provided in a much larger volume of liquid (e.g. 100 µL of solid beads in 1000 µL storage solution). Pipetting this solution up and down mixes the beads into a slurry allowing their transfer to the experimental tube. Also, avoid vigorous mixing during the elution step when the liquid volume is low. Instead, gently tap the tube to agitate the beads within the small volume of sample elution buffer.
- **Blot development:** When analyzing immunocaptured products by Western blotting choose an appropriate method for blot development. The alkaline phosphatase (NBT/BCIP) and horseradish peroxidase (ECL) methods are recommended.

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

9.1 **1X Wash Buffer**

Prepare 1X Wash Buffer by adding 10 mL 10X Wash Buffer to 90 mL nanopure water. Mix gently and thoroughly.

9.2 **1X PBS**

Prepare 1X PBS by adding 10 mL 10X PBS to 90 mL nanopure water. Mix gently and thoroughly.

9.3 **1X Extraction Buffer**

Prepare 1X Extraction Buffer by adding 1 Volume of 10X Extraction Buffer to 9 volumes of 1X PBS. Example, to generate 5 mL 1X Extraction Buffer add 500 µL 10X Extraction Buffer to 4.5 mL 1X PBS.

● Store all diluted reagents at 4ºC after use.

10. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE -

Typical working ranges	
Sample Type	**Range**
Whole cell homogenate	1 – 2 mg

10.1. **Preparation of extracts from cell pellets**

10.1.1. Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. If the adherent cells are detaching, collect both floating cells and remaining adherent cells. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

10.1.2. Rinse cells twice with PBS.

10.1.3. Solubilize cell pellet at 2x107/mL in 1X Extraction Buffer or simply add 9 volumes of 1X Extraction Buffer to one volume of cell pellet, thoroughly mix.

10.1.4. Incubate the mixture on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean microtubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C for 6 months. The sample protein concentration in the extract may be quantified using a protein assay.

10.2. **Preparation of extracts from adherent cells by direct lysis (alternative protocol)**

10.2.1. Remove growth media and rinse adherent cells 2 times in PBS.

10.2.2. Solubilize the cells by addition of Extraction Buffer directly to the plate (use 750 µL - 1.5 mL Extraction Buffer per confluent 15 cm diameter plate).

10.2.3. Scrape the cells into a test tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

10.3. **Preparation of extracts from tissue homogenates**

10.3.1. Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

10.3.2. The protein concentration may be quantified from a small aliquot of the homogenate using a protein assay (e.g. BCA assay).

10.3.3. Suspend the homogenate to a protein concentration of 5.5 mg/mL in PBS.

10.3.4. Solubilize the homogenate by adding 1 volume of 10X Extraction Buffer to 9 volumes of the homogenate, e.g. 20 µL 10X Extraction Buffer to 180 µL homogenate, thoroughly mix.

10.3.5. Incubate the mixture on ice for 20 minutes. Centrifuge at 18,000 x g, for 20 minutes at 4°C. Transfer the supernatant into a clean microtube and discard the pellet. Assay samples immediately or aliquot and store at -80°C.

11. ASSAY PROCEDURE

Note: It is strongly recommended to use a positive control sample along with the tested samples. The sample should be solubilized following the description in sample preparation session. The smallest working amount of beads is around 5 µL of solid beads in a microtube (e.g. 1.5 mL). It is not practical to use a volume smaller than this for the immunocapture procedure.

Prepare all reagents and samples as directed in the previous sections.

11.1 Prepare beads slurry by adding the desired amount of antibody loaded beads (e.g. if 5 µL of solid beads required, add 50 µL of stock bead slurry provided) to 1 mL 1X PBS. Add the appropriate amount of solubilized supernatant (see Typical Sample Dynamic Range table in Sample Preparation Section for reference) to the beads slurry. Optional: add protease inhibitors to reduce sample degradation.

11.2 Allow this mixture to mix for at least 3 hours or overnight (around 16 hours) at 4°C using a tube rotator.

11.3 Collect the beads by centrifugation for 2 minutes at 300 x g.

11.4 Carefully remove the supernatant from the beads.

11.5 Fill the microtube containing the beads with 1X Wash Buffer. Gently tab the microtube to ensure the full suspension of the beads.

11.6 Collect the beads by centrifugation for 2 minutes at 300 x g.

11.7 Repeat steps 11.5 and 11.6 twice for a total of three washes.

11.8 After the last wash, make sure to remove the all of the wash buffer from the tube. The p53 captured on the beads is now ready for elution.

Antigen Elution

11.9 Add 40 µL SDS elution buffer to each 10 µL washed beads. Gently tap the microtube to mix it thoroughly.

11.10 Collect the beads by centrifuge at 12,000 x g for 5 minutes.

11.11 Collect the supernatant from above the beads in a separate microtube and discard the beads. This supernatant contains denatured p53.

11.12 To run the eluted sample in protein electrophoresis, add the correct volume of concentrated Laemmli sample buffer or store the eluted p53 at -80°C.

Sample Analysis

11.13 Samples can now be resolved by electrophoresis. Resolved proteins should be detected by the appropriate method shown below:

Typical working ranges	
Detection Strategy	**Yield of p53**
Gel staining with Coomassie	1 µg +
Gel staining with silver/sypro ruby	10 ng +
Western blotting	1 ng +
Mass spectrometry	Any

11.14 Antibody is crosslinked to the beads therefore no antibody should be present in the sample once eluted from the beads. However, the use of reducing agent such as DTT or β-mercaptoethanol is not recommended.

11.15 Some samples may aggregate on boiling; therefore, 95°C heating or boiling of the sample prior to loading is NOT recommended. Gentle heating at 37°C for 5-10 minutes may be applied to increase the resolution.

12. TYPICAL DATA

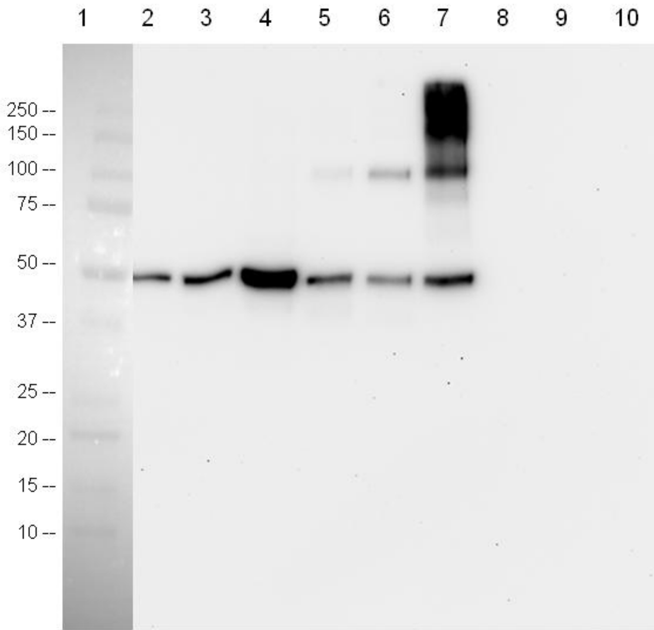


Figure 1. p53 immunocapture kit (ab154470) is compatible with several different extraction methods. Hek293 cells were extracted with lauryl maltoside (lanes 2, 5, 8), RIPA buffer (lanes 3, 6, 9) or SDS (lanes 4, 7, 10). Extracts of whole cells (20 µg, lanes 2-4), one-fifth of immunoprecipitation samples using the p53 immunocapture beads (1 mg extract per 10 µL beads, lanes 5-7), and one-fifth of immunoprecipitation samples using a negative control antibody (ab135397, 1 mg extract per 10 µL beads, lanes 8-10) were analyzed by Western blot using ab32389 anti-p53.

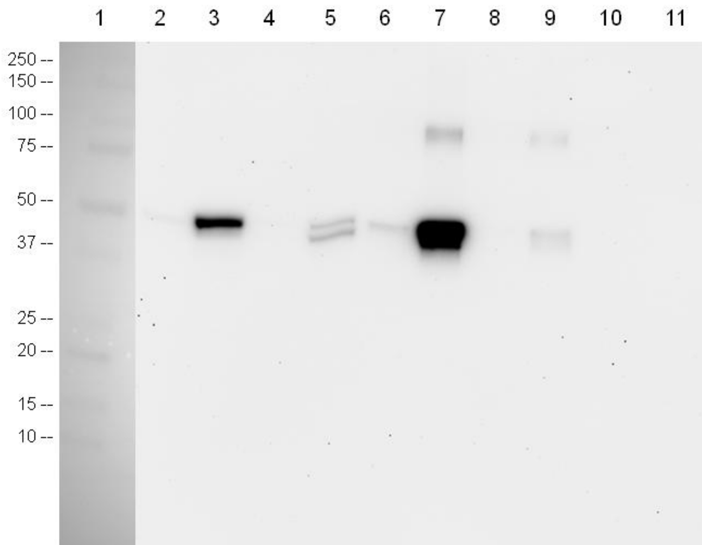


Figure 2. Example of use of p53 immunocapture kit (ab154470) using MCF7 camptothecin-treated cells. MCF7 vehicle-treated cells were extracted with lauryl maltoside (lanes 2, 6) or RIPA buffer (lanes 4, 8, 10). MCF7 cells treated with 1 µM camptothecin were extracted with lauryl maltoside (lanes 3, 7) or RIPA buffer (5, 9, 11). Extracts of whole cells (20 µg, lanes 2-5), one-fifth of immunoprecipitation samples using the p53 immunocapture beads (1 mg extract per 10 µL beads, lanes 6-9), and one fifth of immunoprecipitation samples using a negative control antibody (ab135397, 1 mg extract per 10 µL beads, lanes 10-11) were analyzed by Western blot using ab32389 anti-p53.

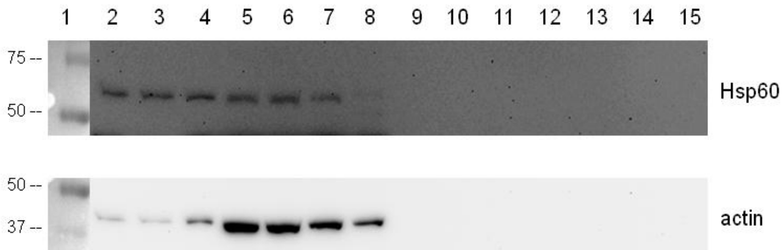


Figure 3. The p53 immunocapture kit is specific to the p53 protein. Hek293 cells were extracted with lauryl maltoside (lanes 2, 9), RIPA buffer (lanes 3, 10) or SDS (lanes 4, 11). MCF7 vehicle-treated cells were extracted with lauryl maltoside (lanes 5, 12) or RIPA buffer (lanes 7, 14). MCF7 cells treated with 1 µM camptothecin were extracted with lauryl maltoside (lanes 6, 13) or RIPA buffer (8, 15). Extracts of whole cells (20 µg, lanes 2-8), and one-fifth of immunoprecipitation samples using the p53 immunocapture beads (1 mg extract per 10 µL beads, lanes 9-15), were analyzed by Western blot using ab46798 anti-Hsp60 and ab46805 anti-muscle actin.

13. ASSAY SPECIFICITY

This immunocapture kit recognizes Human p53 only.

Please contact our Technical Support team for more information.

14. TROUBLESHOOTING

Problem	Solution
Antibody contamination	When eluting, pipette only the liquid part from above the beads. This supernatant can be spun again and recollected to ensure that it is free of beads.
	Do not include reducing agent in sample during electrophoresis. This should maintain any co-eluted antibody at 150 kDa during electrophoresis.
Weak or no signal	Increase the volume of beads added.
	Increase sample/bead ratio.
	Increase the incubation time.
Non-specific bands	Add protease inhibitor when preparing samples.
	Incubate sample/beads mixture at 4°C.
	Add protease inhibitor to the sample/bead mixture during incubation.
	Before eluting the antigen, wash the antigen coated beads thoroughly for a total of three times.

15. NOTES

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