ab178011 – NADH Dehydrogenase (Complex I) Human ELISA Kit

Instructions for Use

For the quantitative measurement of NADH Dehydrogenase (Complex I) in Human cell and tissue extracts.

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

Abcam’s NADH Dehydrogenase (Complex I) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of NADH dehydrogenase protein in Human cell and tissue extracts.

The ELISA employs a labeled capture and detector antibody which immunocaptures the sample analyte in solution. This entire complex (capture antibody/protein/detector antibody) is in turn immobilized in the well by immunoaffinity via the anti-tag antibody. Samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material; the TMB substrate is then added. The reaction is stopped by addition of Stop Solution which stops the color development and completes any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

NADH dehydrogenase (NADH: ubiquinone reductase (H\(^+\)-translocating), Complex I) is the first enzyme of the oxidative phosphorylation (OXPHOS) system within the mitochondrial inner membrane. NADH dehydrogenase is a large protein complex of 950,000 MW made up of 45-46 different subunits. Seven of the subunits of the complex are encoded on mitochondrial DNA (mtDNA), the remaining subunits are nuclear encoded, made in the cytosol and translocated into the organelle for assembly at the inner membrane. The enzyme complex catalyses electron entry from NADH via a flavin (FMN) and several non-heme iron centers. Mutations in mtDNA, or nuclear DNA genes encoding NADH dehydrogenase subunits or assembly factors are a common cause of genetic OXPHOS defects. Mutations or loss of mtDNA may cause enzymatic dysfunction by disrupting enzyme assembly or alternatively by specifically affecting enzymatic activity with no effect on enzyme assembly. NADH
dehydrogenase (like Complex III) has been proposed as a site of superoxide ‘leak’ from the mitochondrial OXPHOS system. Altered functioning and increased superoxide production by this complex has been proposed to contribute to several neurological disorders including Parkinson’s disease. Also there is evidence of NADH Dehydrogenase involvement in diabetes.
2. **ASSAY SUMMARY**

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.

Add standard or sample to appropriate wells.

Add Antibody Cocktail to all wells. Incubate at room temperature.

Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.
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 2-8°C 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**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X NADH Dehydrogenase Capture Antibody</td>
<td>2 x 300 µL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>10X NADH Dehydrogenase Detector Antibody</td>
<td>2 x 300 µL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>HeLa Human Lyophilized Cell Extract</td>
<td>2 Vials</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Antibody Diluent 5B</td>
<td>2 x 3 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>10X Wash Buffer LM</td>
<td>20 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>2X Cell Extraction Buffer LM</td>
<td>10 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>12 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Sample Diluent NS*</td>
<td>12 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Pre-Coated 96 Well Microplate (12 x 8 well strips)</td>
<td>96 Wells</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Plate Seal</td>
<td>1</td>
<td>+2-8°C</td>
</tr>
</tbody>
</table>

* Sample Diluent NS only required for serum and plasma samples.
6. **MATERIALS REQUIRED, NOT SUPPLIED**

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm
- Method for determining protein concentration (BCA assay recommended)
- Deionized water
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4)
- Multi- and single-channel pipettes
- Tubes for standard dilution
- Plate shaker for all incubation steps
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors)

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
GENERAL INFORMATION

8. **TECHNICAL HINTS**

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- The provided 2X Cell Extraction Buffer contains phosphatase inhibitors and protease inhibitor aprotinin. Additional protease inhibitors can be added if required.
- The provided Antibody Diluent and Sample Diluent contain protease inhibitor aprotinin. Additional protease inhibitors can be added if required.
- **This kit is sold based on number of tests.** A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
9. **REAGENT PREPARATION**

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 **1X Cell Extraction Buffer LM**  
Prepare 1X Cell Extraction Buffer LM by diluting 2X Cell Extraction Buffer LM to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer LM combine 5 mL deionized water and 5 mL 2X Cell Extraction Buffer LM. Mix thoroughly and gently. If required protease inhibitors can be added.

9.2 **1X Wash Buffer LM**  
Prepare 1X Wash Buffer LM by diluting 10X Wash Buffer LM with deionized water. To make 50 mL 1X Wash Buffer LM combine 5 mL 10X Wash Buffer LM with 45 mL deionized water. Mix thoroughly and gently.

9.3 **Antibody Cocktail**  
Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5B. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5B. Mix thoroughly and gently.
10. **STANDARD PREPARATION**

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following table describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the HeLa Human Lyophilized Cell Extract standard sample by adding 40 µL water and 160 µL 1X Cell Extraction Buffer LM by pipette. Mix thoroughly and gently. Hold at room temperature for 1 minute. This is the 400 µg/mL **Stock Standard** Solution (see table below). Remaining stock material should be aliquoted and stored at -80°C.

10.2 Label eight tubes with numbers 1 – 8.

10.3 Add 150 µL 1X Cell Extraction Buffer LM into tube numbers 2-8.

10.4 Prepare **Standard #1** by adding 150 µL of the 400 µg/mL Stock Standard Solution to 150 µL of 1X Cell Extraction Buffer LM to tube #1. Mix thoroughly and gently.

10.5 Prepare **Standard #2** by transferring 150 µL from Standard #1 to tube #2. Mix thoroughly and gently.

10.6 Prepare **Standard #3** by transferring 150 µL from Standard #2 to tube #3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes #4 through #7.

10.8 **Standard #8** contains no protein and is the Blank control.
## ASSAY PREPARATION

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Sample to Dilute</th>
<th>Volume to Dilute (µL)</th>
<th>Volume of Diluent (µL)</th>
<th>Starting Conc. (µg/mL)</th>
<th>Final Conc. (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stock</td>
<td>150</td>
<td>150</td>
<td>400</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>Standard #1</td>
<td>150</td>
<td>150</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Standard #2</td>
<td>150</td>
<td>150</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Standard #3</td>
<td>150</td>
<td>150</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>Standard #4</td>
<td>150</td>
<td>150</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>6</td>
<td>Standard #5</td>
<td>150</td>
<td>150</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>7</td>
<td>Standard #6</td>
<td>150</td>
<td>150</td>
<td>6.25</td>
<td>3.125</td>
</tr>
<tr>
<td>8 (Blank)</td>
<td>none</td>
<td>0</td>
<td>150</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

![Image of assay preparation](image_url)
11. SAMPLE PREPARATION

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Range (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa cell lysate</td>
<td>3 – 200</td>
</tr>
<tr>
<td>HepG2 cell lysate</td>
<td>1.5 – 75</td>
</tr>
<tr>
<td>143B cell lysate</td>
<td>3 – 200</td>
</tr>
</tbody>
</table>

11.1 Preparation of extracts from cell pellets

11.1.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.1.2 Rinse cells twice with PBS.

11.1.3 Solubilize pellet at 2x10⁷ cell/mL in chilled 1X Cell Extraction Buffer LM.

11.1.4 Incubate on ice for 20 minutes.

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

11.1.6 Transfer the supernatants into clean tubes and discard the pellets.

11.1.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.1.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer LM.

11.2 Preparation of extracts from adherent cells by direct lysis (alternative protocol)

11.2.1 Remove growth media and rinse adherent cells 2 times in PBS.
11.2.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer LM directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer LM per confluent 15 cm diameter plate).

11.2.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

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

11.2.5 Transfer the supernatants into clean tubes and discard the pellets.

11.2.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.2.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer LM.

11.3 Preparation of extracts from tissue homogenates

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

11.3.2 Homogenize 100 to 200 mg of wet tissue in 500 µL - 1 mL of chilled 1X Cell Extraction Buffer LM. For lower amounts of tissue adjust volumes accordingly.

11.3.3 Incubate on ice for 20 minutes.

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

11.3.5 Transfer the supernatants into clean tubes and discard the pellets.

11.3.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.3.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer LM.
12. **PLATE PREPARATION**

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.
13. **ASSAY PROCEDURE**

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Substrate to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

*Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:*
### ASSAY PROCEDURE

<table>
<thead>
<tr>
<th>Mode</th>
<th>Kinetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength:</td>
<td>600 nm</td>
</tr>
<tr>
<td>Time:</td>
<td>up to 15 min</td>
</tr>
<tr>
<td>Interval:</td>
<td>20 sec - 1 min</td>
</tr>
<tr>
<td>Shaking:</td>
<td>Shake between readings</td>
</tr>
</tbody>
</table>

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.9 Analyze the data as described below.
14. **CALCULATIONS**

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
15. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

<table>
<thead>
<tr>
<th>Conc. (μg/mL)</th>
<th>O.D. 450 nm</th>
<th>Mean O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0597</td>
<td>0.0673</td>
</tr>
<tr>
<td>3.13</td>
<td>0.1342</td>
<td>0.1324</td>
</tr>
<tr>
<td>6.25</td>
<td>0.2119</td>
<td>0.2144</td>
</tr>
<tr>
<td>12.50</td>
<td>0.3465</td>
<td>0.3231</td>
</tr>
<tr>
<td>25.00</td>
<td>0.5954</td>
<td>0.5661</td>
</tr>
<tr>
<td>50.00</td>
<td>1.1256</td>
<td>1.1118</td>
</tr>
<tr>
<td>100.00</td>
<td>2.1376</td>
<td>2.1259</td>
</tr>
<tr>
<td>200.00</td>
<td>3.6017</td>
<td>3.6492</td>
</tr>
</tbody>
</table>

**Figure 1.** Example of NADH Dehydrogenase standard curve. The NADH Dehydrogenase standard curve was prepared as described in Section 10 using HeLa cell extract. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.
16. **TYPICAL SAMPLE VALUES**

**SENSITIVITY –**
The calculated minimal detectable (MDD) dose is 0.43 μg/mL of HeLa cell extract. The MDD was determined by calculating the mean of zero standard replicates (n=32) and adding 2 standard deviations then extrapolating the corresponding concentrations.

**RECOVERY –**
(Sample spiking in representative sample matrices)

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Medium (10F HGDMEM)</td>
<td>66</td>
<td>64-67</td>
</tr>
<tr>
<td>10% Fetal Bovine Serum</td>
<td>74</td>
<td>70-77</td>
</tr>
<tr>
<td>10% Normal Human Serum</td>
<td>110</td>
<td>108-111</td>
</tr>
</tbody>
</table>

**LINEARITY OF DILUTION –**

<table>
<thead>
<tr>
<th>Dilution Factor</th>
<th>HepG2 Lysate (µg/mL)</th>
<th>Interpolated value (ng/mL)</th>
<th>% Expected Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>75</td>
<td>202.4</td>
<td>100.0</td>
</tr>
<tr>
<td>2</td>
<td>37.5</td>
<td>103.1</td>
<td>101.9</td>
</tr>
<tr>
<td>4</td>
<td>18.75</td>
<td>53.2</td>
<td>105.1</td>
</tr>
<tr>
<td>8</td>
<td>9.375</td>
<td>27.0</td>
<td>106.7</td>
</tr>
<tr>
<td>16</td>
<td>4.6875</td>
<td>14.0</td>
<td>110.9</td>
</tr>
<tr>
<td>32</td>
<td>2.34375</td>
<td>7.4</td>
<td>117.6</td>
</tr>
</tbody>
</table>

**PRECISION –**
Mean coefficient of variations of interpolated values from 3 concentrations of HepG2 cell lysate within the working range of the assay.

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.7</td>
<td>5.9</td>
</tr>
</tbody>
</table>
**Figure 2.** Titration of HepG2 cell lysate within the working range of the assay. Background-subtracted data values from triplicate measurements (mean +/- SD) are graphed.

**17. ASSAY SPECIFICITY**

This assay recognizes native NADH Dehydrogenase protein.

**Figure 3.** Comparison of NADH Dehydrogenase expression in 143B wildtype (WT) and two clones (3B10 and 3F5) of 143B-derived Rho<sup>0</sup> (mitochondrial DNA-depleted) cells. Background-subtracted data values from triplicate measurements of three lysate concentrations (200, 100 and 50 µg/mL) are graphed as mean +/- SD.
Figure 4. Quantification of NADH Dehydrogenase expression in 143B wildtype (WT) and two clones (3B10 and 3F5) of 143B-derived Rho<sup>0</sup> (mitochondrial DNA-depleted) cells. The concentrations of NADH Dehydrogenase were interpolated from data values shown in Figure 3 using NADH Dehydrogenase standard curve of HeLa cell extract, corrected for sample dilution, and graphed in percent relative to NADH Dehydrogenase expression in HeLa cell extract. The concentration of NADH Dehydrogenase in both Rho<sup>0</sup> cell lines was less than 1% of the concentration in the WT 143B cells.

18. **SPECIES REACTIVITY**

This kit detects NADH Dehydrogenase in Human cell and tissue samples only.

Serum and plasma samples have not been tested with this kit.

Please contact our Technical Support team for more information.
### Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inaccurate Pipetting</td>
<td>Check pipettes</td>
<td></td>
</tr>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation</td>
</tr>
<tr>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
<td></td>
</tr>
<tr>
<td>Incubation times with TMB too brief</td>
<td>Ensure sufficient incubation time until blue color develops prior addition of Stop solution</td>
<td></td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the ELISA kit</td>
<td>Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.</td>
</tr>
</tbody>
</table>
20. **NOTES**
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