ab119632

PhosphoTracer

IKKα (pS176/180)

ELISA Kit

Instructions for Use

For the semi-quantitative measurement of IKKα (pS176/180) concentrations in cell culture extracts

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

IKKα is part of the IκB kinase (IKK) complex, which consists of three IKK subunits; IKKα, IKKβ and IKKγ. IKKα and IKKβ are catalytic subunits of the complex, while IKKγ serves a regulatory function. The IKK complex plays an important role in regulating NF-κB. Many factors that activate NF-κB operate through a common pathway based on regulation of I-κB. A key step in this pathway involves activation of the IKK complex, which in turn phosphorylates I-κB, leading to its dissociation from NF-κB and subsequent proteasome-mediated degradation. Phosphorylation of Ser176 and Ser180 in the activation loop of IKKα and Ser177 and Ser181 in IKKβ results in conformational change and activation of the complex.
**Specificity:**

Abcam’s PhosphoTracer IKKα assay kits detect endogenous levels of IKKα (GenBank Accession NP_001269) in cellular lysates. Phospho-IKKα assay kits detect IKKα only when phosphorylated at Ser176/180.

**Species cross-reactivity:**

Tested: Human, Mouse

Expected: Rat

Other species should be tested on a case-by-case basis.
Figure 1. As shown, using the IKKα assay kits, a significant stimulation of IKKα phosphorylation at Ser176/180 is detected in HeLa cells treated with TNFα for 5 minutes (+) compared with untreated cells (-), while no change in total IKKα levels is observed over this time period.
Figure 2. A dilution series of recombinant active IKKα was prepared by diluting to various concentrations in Lysis Mix, containing 0.1% BSA. These dilution series were analyzed using the phospho-IKKα assay. Limit of detection of active rhIKKα was around 250 pg/mL. No cross-reactivity with active rhIKKβ was detected over this concentration range.
2. Assay Principle

PhosphoTracer assays use a traditional immuno-sandwich format, but with a major difference. For PhosphoTracer assays, both the analyte and the assay reagents are added to the PhosphoTracer assay microplate at the same time. After a short incubation period, unbound assay reagents and analytes are washed away, and immuno-complexes containing both antibodies are detected. The process can take as little as 60 minutes to complete.

PhosphoTracer also allows a higher degree of assay flexibility. In contrast to other ELISA formats, no antibodies are present on the assay microplate itself, so assays for several different targets can be performed in different wells on the same microplate. Simply mix the lysate with your target reagents of choice, using the microplate configuration of your choice. For example, a cellular lysate can be analyzed on the same assay microplate in parallel for p38-MAPK phosphorylation, ERK phosphorylation, and JNK phosphorylation, giving fast, accurate and quantifiable information on key cell signaling events.

A whole new way of performing cellular assays, PhosphoTracer takes the hard work out of running a standard ELISA, while still giving the high quality results expected from a sandwich immunoassay. Fully self-contained kits are supplied in convenient
96-well packs. Simple to use and highly sensitive, PhosphoTracer kits are designed to get results, fast.

3. Assay Summary

Add lysates to PhosphoTracer assay microplate.

\[\downarrow\]

Add Antibody Mix to microplate, incubate 1 hour.

\[\downarrow\]

Wash microplate.

\[\downarrow\]

Add Substrate Mix to microplate. Cover with foil, incubate 10 minutes.

\[\downarrow\]

Read microplate
# 4. Kit Contents

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Buffer (5X)</td>
<td>1 x 15 mL</td>
</tr>
<tr>
<td>Enhancer Solution</td>
<td>1 x 1 mL</td>
</tr>
<tr>
<td>ADHP Dilution Buffer</td>
<td>1 x 15 mL</td>
</tr>
<tr>
<td>ADHP (100X)</td>
<td>1 x 120 µL</td>
</tr>
<tr>
<td>Wash Buffer (10X)</td>
<td>1 x 15 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 2 mL</td>
</tr>
<tr>
<td>Assay Control Lysate (lyophilized)</td>
<td>1 x 0.25 mL</td>
</tr>
<tr>
<td>96-well PhosphoTracer assay plate (stripwell)</td>
<td>1</td>
</tr>
<tr>
<td>Adherent plate seals</td>
<td>2</td>
</tr>
<tr>
<td>Number of vials per target</td>
<td>For ab119674 to ab119679</td>
</tr>
<tr>
<td>----------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td><strong>Capture Antibody Reagent</strong></td>
<td>1 x 1 mL</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Detection Antibody Reagent</strong></td>
<td>1 x 1 mL</td>
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<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**PhosphoTracer assay microplates**

Only use PhosphoTracer assay microplates for PhosphoTracer assays. PhosphoTracer assay microplates are specifically designed to work with PhosphoTracer assays, and cannot be substituted with other 96-well microplates. The PhosphoTracer assay microplate is highly optimized to form an active surface for the capture of immunocomplexes that are formed as part of the PhosphoTracer assay procedure.

After opening, the PhosphoTracer microplate can be stored at 4°C. The microplate should be allowed to equilibrate to room temperature prior to opening the pouch, to minimize condensation forming in the wells.

PhosphoTracer assay microplates are supplied in convenient 8-well strips. Only use the amount of strips required for the assay. Remove strips from the frame that are not needed, place back in their foil pouch, seal with tape, and store at 4°C. Unused wells should be used within 1 month of opening the microplate foil bag.

**Antibody Mix**

The Antibody Mix is comprised of a 1:1 mixture of the Capture Antibody Reagent and the Detection Antibody Reagent, and contains all of the antibodies necessary for the assay.
The Capture Antibody Reagent contains the capture antibody, and the Detection Antibody Reagent contains the detection antibody. The antibody reagents need to be mixed prior to use, to form the Antibody Mix.

The Antibody Mix can be prepared by adding an equal volume of Capture Antibody Reagent and Detection Antibody Reagent, and mixing by inversion prior to each experiment. Alternatively, simply combine and mix all of the contents of the Capture Antibody Reagent and Detection Antibody Reagent before the first use of the kit, to yield 6 mL of ready-to-use Antibody Mix.

**Lysis Mix**

The Lysis Mix is a combination of the Lysis Buffer and Enhancer Solution. The 5X Lysis Buffer contains a combination of detergents, phosphatase inhibitors, salts and buffers. 5X Lysis Buffer is supplemented with Enhancer Solution to yield a versatile lysis solution that can be applied to many cells and tissues.

The *Lysis Mix Concentrate* is used to lyse cells in the presence of culture medium, and is typically used to lyse non-adherent cells.

The *Lysis Mix* is used to lyse cells after the removal of culture medium, and is typically used to lyse adherent cells, or non-adherent cells that have been harvested by centrifugation. Lysis Mix is prepared by simply diluting Lysis Mix Concentrate 5-fold with water.
Lysis Mix should be used as the diluent for any dilution of cellular lysates that are required.

Supplementing Lysis Mix with extra components (e.g. protease inhibitors, chelating agents, detergents) should be tested on a case-by-case basis for compatibility with PhosphoTracer assays.

**Assay Control Lysates**

Assay Control Lysates are prepared from various cell types, which have been cultured and prepared to optimize the activation of the intracellular pathway of interest.

The Assay Control Lysates are intended for use as an assay positive control only, and should not be used for the absolute quantification of a particular protein or phosphorylated target. In combination with negative control wells containing Lysis Mix only, the Assay Control Lysates can be used to give an indication of the expected signal range for a given assay.

The controls are supplied lyophilized, and should be reconstituted with 250 µL of either dd H₂O or high quality purified H₂O. If required, control lysates can be further diluted with Lysis Mix, and frozen in aliquots for subsequent use.
Wash Buffer

The Wash Buffer, supplied as a 10X concentrate, is used for washing the PhosphoTracer assay microplate. It is a simple mix of buffer, salts and mild detergent. Alternatively, a PBS/0.05% (v/v) Tween-20 solution may be substituted as a wash solution.

If washing wells with a microplate washer, use 3x washes with a 10 second mixing cycle, with a PBS/0.05% (v/v) Tween-20 wash solution.

Substrate Mix

The Substrate Mix is a combination of 10-Acetyl-3,7-dihydroxyphenoxazine (ADHP), a sensitive substrate for HRP, and ADHP Dilution Buffer, a stabilized H2O2 solution. In the presence of H2O2, HRP converts ADHP to the fluorogenic product, resorufin. Resorufin is a highly fluorescent molecule, and should be used with excitation filters in the range of 530-540nm, with bandwidths ≤ 30nm. The emission filter should be in the range of 590-600nm, with bandwidths ≤ 30nm.

The signal in the wells should be developed for around 10 minutes. Best results will be obtained if the microplates are developed in the dark, e.g. by covering the microplate with foil.
Stop Solution

The Stop Solution is used for stopping HRP-mediated conversion of ADHP to resorufin. Once added to the wells, signal in the wells is stable for 60 minutes. However, ADHP remaining in the wells is still light-sensitive, and should be handled under low-light conditions until the microplate is read.

5. Storage and Handling

- **Unopened kit:** Store all kit reagents at 4°C. Do NOT freeze the kits.

- **Opened/Reconstituted:** Store at 4°C after opening, until expiry date. For the PhosphoTracer assay microplate: Return unused wells to foil pouch with desiccant pack, and seal with tape. May be stored at 4°C for up to 1 month after opening. For the assay control lysate: aliquot and store at -20°C for up to 1 month.
6. Buffer Preparation

*Note: Bring all reagents to room temperature before use. Avoid vortexing the Capture Antibody Reagent or Detection Antibody Reagent, as vigorous mixing can damage some antibodies.

<table>
<thead>
<tr>
<th>Item</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhancer Solution</td>
<td>Supplied as a concentrate. Precipitation will occur during storage at 4°C, which is normal. To re-dissolve, warm to 37°C and mix by inversion prior to use. Alternatively, Enhancer Solution can be stored at room temperature with no loss in activity.</td>
</tr>
<tr>
<td>Lysis Mix Concentrate</td>
<td>Prepare Lysis Mix Concentrate by diluting Enhancer Solution 10-fold in 5X Lysis Buffer (e.g. mix 900 µL 5X Lysis Buffer and 100 µL Enhancer Solution). Lysis Mix Concentrate is used to lyse cells directly in cell culture medium. Prepare immediately prior to use. Discard unused Lysis Mix Concentrate.</td>
</tr>
<tr>
<td><em><em>Antibody Mix</em> (Capture Antibody Reagent + Detection Antibody Reagent)</em>*</td>
<td>Prior to each experiment, prepare by adding an equal volume of Capture Antibody Reagent and Detection Antibody Reagent, and mixing by inversion. Prepare enough to use 50 µL/well (e.g. for a single 8-well strip, prepare 400 µL Antibody Mix).</td>
</tr>
<tr>
<td>---</td>
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</tr>
<tr>
<td><strong>Wash Buffer</strong></td>
<td>Dilute 10X Wash Buffer 10-fold with either dd H₂O or high quality purified H₂O. (e.g. mix 1 mL of 10X Wash Buffer and 9 mL water). Prepare enough for 3 separate exchanges with 200 µL of 1X Wash Buffer per well.</td>
</tr>
<tr>
<td><strong>Assay Control Lysates</strong></td>
<td>The controls are supplied lyophilized, and should be reconstituted with 250 µL of either dd H₂O or high quality purified H₂O. If required, control lysates can be further diluted with Lysis Mix. Once reconstituted, aliquot and store at -20°C for up to 1 month.</td>
</tr>
</tbody>
</table>
| Substrate Mix | Allow ADHP to thaw prior to use – it is solid at 4°C.  
|              | Avoid preparation or use under bright lights or direct sunlight – ADHP is sensitive to bright light.  
|              | To prepare the Substrate Mix, dilute ADHP 100-fold with ADHP Dilution Buffer A (e.g. add 20 μL ADHP to 2 mL of the ADHP Dilution Buffer) immediately prior to use. The Substrate Mix is then ready to use. Prepare enough to use 100 μL/well.  
|              | For best results we recommend preparing the Substrate Mix immediately prior to use. It is not stable for long periods once mixed. |
7. Lysate Preparation

These lysate preparations require the preparation of cellular lysates in a tissue culture microplate. The lysate is subsequently transferred to a PhosphoTracer microplate for the assay. The exception to this is the all-in-one well assays which avoid the lysate transfer steps.

**Adherent cells**

1. Remove any media and cellular treatments from the cells – optionally, wash cells with PBS.

2. For cells cultured in 96-well microplates, lyse the cells with 100 µL* of freshly prepared Lysis Mix, with shaking (~300 rpm) at room temp for 10 minutes.

    *Lysis volume should be adjusted depending on the desired lysate concentration. Lysates in the range of 0.1 - 0.5 mg/mL protein are usually sufficient. However, preparing more concentrated lysates can help with the detection of low abundance analytes.

**Proceed to Section 8.**
Non-adherent cells

1. Harvest cells by centrifugation, and resuspend at an appropriate density in HBSS* containing 5% FBS. A cell density that yields cellular lysate at a protein concentration of 0.1 - 0.5 mg/mL is suitable for many cell lines.

*Avoid resuspending cells in cell culture medium for the assay, as components of common media can interfere with the assay. For example RPMI contains biotin that will interfere with the assay. If the use of media is necessary for the cells, after treatment the cells can be harvested by centrifugation, and washed with PBS or HBSS prior to lysis.

2. Return cells to a 37°C incubator for 1-2 hours. For certain pathways, this can allow handling-mediated pathway activation to subside. This step is optional, and depends on the activation status of your cells following re-suspension.

3. At the completion of the treatment, lyse cells with 1/5th final volume of Lysis Mix Concentrate, with shaking (~300 rpm) at room temp for 10 min (e.g. for 40 µL of cells, use 10 µL of Lysis Mix Concentrate).
Alternatively cells can be harvested by centrifugation and lysed with Lysis Mix.

Proceed to Section 8.

All-in-one well assays

This method avoids lysate transfer steps

1. Harvest cells by centrifugation, and resuspend at an appropriate density in HBSS* containing 5% FBS. A cell density that yields 10,000-25,000 cells/well is suitable for the analysis of many cell lines.

*Avoid resuspending cells in cell culture medium for the assay, as components of common media can interfere with the assay. For example RPMI contains biotin that will interfere with the assay. If the use of media is necessary for the cells, after treatment the cells can be harvested by centrifugation, and washed with PBS or HBSS prior to lysis.

2. Determine desired number of PhosphoTracer microplate strips. Remove unused strips from frame and return to storage pouch and seal.

3. Add 20 µL cells/well to the PhosphoTracer assay microplate.
4. Return cells to a 37°C incubator for 1 - 2 hours. For certain pathways, this can allow handling-mediated pathway activation to subside. This step is optional, and depends on the activation status of your cells following re-suspension.

5. Add 20 µL treatment/well to cells (e.g. 2x agonists and/or antagonists) for the desired time period. The final volume in wells prior to lysis should be 40 µL.

6. At the completion of the treatment, lyse cells with 10 µL Lysis Mix Concentrate, with shaking (~300 rpm) at room temp for 10 minutes.

   Proceed to Section 8, step 3.
8. PhosphoTracer Assay Protocol

For All-in-one well assays proceed directly to step 3.

1. Determine desired number of PhosphoTracer microplate strips. Remove unused strips from frame and return to storage pouch and seal.

2. Add 50 µL/well of lysate to the PhosphoTracer microplate.

3. Add 50 µL/well Lysis Mix (negative control) and Control Lysates (positive control) to separate wells for assay controls if desired.

4. Add 50 µL/well of Antibody Mix to the wells. Cover the microplate with adhesive seal and incubate for 1 hr at room temp on a microplate shaker (~300 rpm).

5. Wash wells with 200 µL/well 1X Wash Buffer (repeat 3 times). After final wash, remove any remaining wash solution from wells.

6. Immediately prior to use, prepare Substrate Mix. Add 100 µL/well of Substrate Mix. Cover microplate with foil, and incubate for 10 minutes at room temp on a microplate shaker (~300 rpm).
Substrate Mix should be prepared immediately prior to use, preferably while microplate is washing, but do not allow microplate to dry. If necessary, leave microplate in 1X Wash Buffer for up to 30 minutes, until Substrate Mix has been prepared.

7. Add 10 µL/well Stop Solution, and mix briefly (5 - 10 sec) on a microplate shaker.

8. Read fluorescence signal with a PhosphoTracer compatible filter set.
9. Data Analysis

To analyze the data, calculate the averaged counts for untreated and treated cells. We recommend using at least duplicate wells (n=2) to calculate a response.

Dose response and dose inhibition curves can be fitted to 4 parameter non-linear regression equations. These types of regression analyses output key parameters such as EC$_{50}$ (or IC$_{50}$), Min and Max signals, and Hillslope factors.

Ensure that samples readings are within the linear range of the assay. This can vary based on reader performance, and analyte concentration. If a lysate sample generates a signal outside the linear range, the lysate samples should be diluted with Lysis Mix and re-assayed. Absolute fluorescent units will vary from reader to reader.
10. **Procedure Limitations**

- Variations in general operator-related procedures, such as pipetting, washing, and incubation times, can cause variation in the final signal.

- The assay is designed to work for the detection of endogenous cellular proteins across a wide variety of cell lines. However, until each cell line in particular is tested, the possibility of the presence of interfering factors cannot be excluded.

- Users should ensure that their cell line has measurable levels of the pathway of interest. Expression levels of signaling proteins in different cell types vary widely.
11. Technical Hints and Troubleshooting

- **General cell handling**

  Cells should be harvested from flasks for seeding into microplates when approximately 70-90% confluent. The cells should be detached from the flasks using mild conditions (such as trypsin-free cell dissociation solutions where possible), accurately counted, and diluted to the appropriate density in fresh media. If using adherent cells, allow time for cells to regain full signaling capacity after harvesting.

- **Compatible cell types**

  The assay can be used for many adherent and non-adherent cell types, including transfected cell lines and primary cells. However, because kinase expression and phosphorylation conditions can vary from one cell line to another, some cells may be more amenable to particular assays than others. Parameters such as stimulation time and cell number should be optimized for each cell line used.

  Cells over-expressing a receptor of interest have been shown to elicit good phosphorylation responses. When using over-expressed intracellular targets, ensure the expressed target is full-length to ensure correct binding of assay antibodies. The concentration of cell lysate should be
optimized to ensure the signal is within the working range of the assay.

- **Lysate viscosity and handling**

Lysing cells with Lysis Mix can yield lysates that are viscous and difficult to handle, particularly when concentrated lysates are required. Care should be taken when transferring lysates, to minimize pipetting-related variability.

To avoid this problem when using non-adherent cells, the cells can be transferred to the PhosphoTracer assay microplate immediately prior to lysis, and subsequently lysed directly in the assay microplate (e.g. transfer 40 µL cells/well to an PhosphoTracer assay microplate, and lyse with 10 µL 5X Lysis Mix Concentrate – see Kit Contents for preparation).

Alternatively, use the all-in-one-well assay procedure.

- **Assaying for multiple targets from a single lysate**

One of the features of PhosphoTracer protocols is the use of a common assay microplate for all assays. Therefore, a cellular lysate can be transferred to several replicate wells, and analyzed in parallel. This can enable a particular lysate to be sampled for both total and phosphorylated target on
the same assay microplate, or for several assay targets on
the same microplate.

• **Assay scalability**

The PhosphoTracer assay is optimized for equal 50 µL
volumes of both the analyte and the Antibody Mix. However,
in cases where lysate is limiting, as little as 25 µL of lysate
can be used, in combination with 50 µL of Antibody Mix.

• **Assay incubation times**

The general assay incubation times that are recommended
are 1 hour after the addition of Antibody Mix, and 10 minutes
for the development of the Substrate Mix. Longer
incubations (up to overnight) may be more convenient for
certain assays, and can enhance sensitivity in some cases.

• **Buffer compatibility**

PhosphoTracer assays are compatible with most cell culture
media and reagents, however there are some exceptions.
Media that contain biotin (e.g. RPMI) will reduce assay
sensitivity. When it is necessary to use a media such as
RPMI for growing cells, they should be harvested and re-
suspended in HBSS or a similar buffer, prior to the assay.
Supplementing Lysis Mix with extra components (e.g. protease inhibitors, chelating agents, detergents) is typically okay, but should be tested on a case-by-case basis for compatibility with PhosphoTracer assays. For example, strong detergents such as SDS can denature proteins, and is not recommended for use with PhosphoTracer assays.

• **Lysis Buffer Formulation**

  1X Lysis buffer contains the following phosphatase inhibitors:
  
  - Sodium pyrophosphate
  - Sodium fluoride
  - Sodium orthovanadate

  The addition of other components to the Lysis Mix should be tested on a case-by-case basis.

• **Phosphoprotein analysis**

  Typical analysis of phosphoproteins will involve analysis of the up regulation of a phospho-epitope on a protein, in conjunction with analysis of the protein itself. This ensures that any increase in signal is due to specific phosphorylation, and not just accumulation of the protein itself.
Demonstration of specific phosphorylation of a particular protein or pathway can be typically achieved through:

— Use of specific agonists that result in activation (phosphorylation) of the pathway/protein of interest.
— Use of specific antagonists that prevent activation or deactivate the pathway/protein of interest.

Assay Control Lysates prepared from various cell types are supplied with each kit. The Assay Control Lysates are intended for use as an assay positive control only, and should not be used for the absolute quantification of a particular protein or phosphorylated target. In combination with negative control wells containing Lysis Mix only, the Assay Control Lysates can be diluted to give an indication of the expected signal range (high/medium/low) for a given assay.

- **Assay Specificity**

PhosphoTracer assays are sandwich immunoassays that use both an antibody against a specific phospho-epitope, and a distal epitope on the same protein. Therefore, only analyte that has both antibodies bound can contribute to signal.
All antibodies are rigorously tested for specificity and performance. Furthermore, the use of a 2-antibody format gives extra assurance against the possibility of signal generated from non-specific binding.

- **PhosphoTracer assay microplates**

PhosphoTracer assay microplates are specific to PhosphoTracer assays, and cannot be substituted with other 96-well microplates. The PhosphoTracer assay microplate is highly optimized to form an active surface for the capture of immuno-complexes that are formed as part of the PhosphoTracer assay procedure. PhosphoTracer assay microplates are supplied in 8-well strips. Remove strips from the frame that are not needed, place back in their foil pouch, seal with tape, and store.

After opening, the PhosphoTracer microplate can be stored at 4°C. The microplate should be allowed to equilibrate to room temperature prior to opening the pouch, to minimize condensation forming in the wells. Unused wells should be used within 1 month of opening the microplate foil bag.
• **ADHP HRP substrate**

The HRP substrate used in PhosphoTracer assays is a combination of 10-Acetyl-3,7-dihydroxyphenoxazine (ADHP), a sensitive substrate for HRP, and ADHP Dilution Buffer, a stabilized \( \text{H}_2\text{O}_2 \) solution. In the presence of \( \text{H}_2\text{O}_2 \), HRP converts ADHP to the fluorogenic product, resorufin. Resorufin is a highly fluorescent molecule, and should be used with excitation filters in the range of 530 - 540nm, with bandwidths \( \leq 30\text{nm} \). The emission filter should be in the range of 590 - 600nm, with bandwidths \( \leq 30\text{nm} \).

The signal in the wells should be developed for around 10 minutes. Best results will be obtained if the microplates are developed in the dark, e.g. by covering the microplate with foil. The Stop Solution provided can be used for stopping HRP-mediated conversion of ADHP to resorufin. Once added to the wells, signal in the wells is stable for 60 minutes. However, ADHP remaining in the wells is still light-sensitive, and should be handled under low-light conditions until the microplate is read.

• **Cell Lysis**

The PhosphoTracer Lysis buffer that is supplied, is a 5X Lysis stock containing a combination of detergents, phosphatase inhibitors, salts and buffers. 5X Lysis Buffer is
supplemented with Enhancer Solution to yield a versatile lysis solution that can be applied to many cells and tissues. Supplementing Lysis Mix with extra components (e.g. protease inhibitors, chelating agents, detergents) should be tested on a case-by-case basis for compatibility with PhosphoTracer assays.

Lysing cells can yield lysates that are viscous and difficult to handle, particularly when concentrated lysates are required. Care should be taken when transferring lysates, to minimize pipetting-related variability.

To avoid this problem when using non-adherent cells, the cells can be transferred to the PhosphoTracer assay microplate immediately prior to lysis, and subsequently lysed directly in the assay microplate (e.g. transfer 40 µL cells/well to an PhosphoTracer assay microplate, and lyse with 10 µL 5X Lysis Mix Concentrate - see PhosphoTracer product manual for details).

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
UK, EU and ROW
Email: technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

US, Canada and Latin America
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

China and Asia Pacific
Email: hk.technical@abcam.com
Tel: 108008523689 (中國聯通)
www.abcam.cn

Japan
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp

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