ab140363
Cell Cycle In-Cell ELISA Kit (Fluorescent)

Instructions for use:

For measuring in high throughput, levels of cell cycle marker proteins Cdk2 phosphorylated at tyrosine 15 and Histone H3 phosphorylated at serine 10 in human and mouse cell lines.

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

Version 3 Last Updated 24 September 2015

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1. BACKGROUND

Principle: ab140363 is an In-Cell ELISA (ICE) assay kit that employs quantitative immunocytochemistry to measure levels of Cdk2 protein phosphorylated Tyr15 and Histone H3 protein phosphorylated Ser10 levels in cultured cells. Cells are fixed in a microplate and targets of interest are detected with highly specific, well-characterized antibodies. Relative target levels are quantified using secondary antibodies conjugated to either horseradish peroxidase (HRP) or alkaline phosphatase (AP) which generate signal through the use of two spectrally distinct fluorogenic substrates. Fluorescence is measured using a standard fluorescent spectrophotometer and relative levels of target proteins are quantified. Optionally, antibody signal intensity can be normalized to the total cell amount using Janus Green stain.

Background: The Cdk2 (pTyr15) + Histone H3 (pSer10) In-Cell ELISA Kit (Fluorescent) (ab140363) is designed to study cell cycle effects in response to various stimuli. Monoclonal antibodies specific to Cdk2 (pTyr15) and Histone H3 (pSer10) are used in this high-throughput duplexing plate-based assay. Cdk2 (pTyr15) is elevated in G1/S phase of the cell cycle and Histone H3 (pSer10) is elevated in G2/M phase.

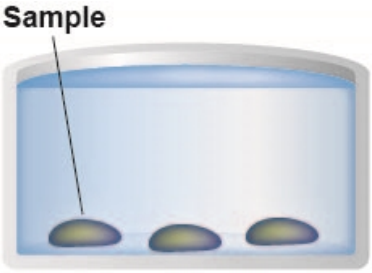
Cyclin-dependent kinase 2 (Cdk2) is a nuclear protein kinase that functions in the G1/S phase of the cell cycle. Inhibitory phosphorylation occurs on residues Thr14 and Tyr15; activation of Cdk2 includes dephosphorylation of these residues by cdc25. Cdk2 can form a complex with Cyclin A, D or E. Phosphorylation of Cdk2 at Tyr15 indicates that a cell is at the G1/S transition.

Histone H3 is one of the four core histone proteins (H2A, H2B, H3 and H4) that pack DNA in nucleosomes. Post-translational modifications of histones include phosphorylation and acetylation and are important for chromatin assembly and gene expression. Phosphorylation of Histone H3 at Ser10 is tightly correlated with chromosome condensation during mitosis. Hence, Histone H3 pSer10 signal indicates a mitotic cell with condensed DNA.

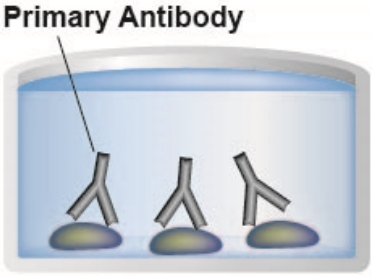
In-Cell ELISA (ICE) technology is used to perform quantitative immunocytochemistry of cultured cells using enzyme linked secondary antibodies and fluorogenic substrates. The technique generates

quantitative data with specificity similar to western blotting, but with much greater quantitative precision and higher throughput due to the greater dynamic range and linearity of fluorescence detection as well as the ability to run up to 96 samples in parallel. Because the Cdk2 (pTyr15) antibody is a rabbit antibody and the Histone H3 (pSer10) antibody is a mouse antibody, they can be measured simultaneously in the same well using the cocktail of provided primary antibodies, species-specific secondary antibodies and fluorogenic substrates. This method rapidly fixes the cells in situ, stabilizing the in vivo levels of proteins and their post-translational modifications, and thus eliminating changes during sample handling, such as in the preparation of protein extracts. Finally, the Cdk2 (pTyr15) and Histone H3 (pSer10) signals can be normalized to cell amount, measured by the provided Janus Green whole cell stain, to further increase the assay precision.

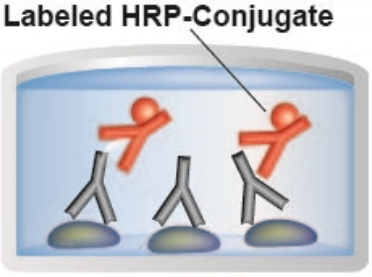
2. ASSAY SUMMARY



Seed cells and incubate overnight. Apply treatment activators or inhibitors. Fix cells with Fixing Solution. Incubate at room temperature. Add Quenching Buffer. Incubate at room temperature. Add Blocking Solution. Incubate at 37ºC.



Add prepared primary antibody to each well used. Incubate at room temperature.



Empty and wash each well. Add prepared secondary antibody. Incubate at room temperature.

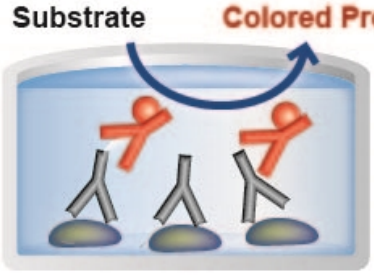


Image plate and analyze data.

If desired, stain with Janus Green and measure relative cell seeding density in a microplate spectrophotometer or IR imager.

Calculate ratios and perform data analysis.

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. STORAGE AND STABILITY

Store kit at 4ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. 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.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X Phosphate Buffered Saline (PBS)	100 mL	4°C
100X Triton X-100 (10% solution)	500 µL	4°C
400X Tween-20 (20% solution)	2 mL	4°C
10X Blocking Solution	10 mL	4°C
100X Anti- Cdk2 (pTyr15) Primary Antibody (Rabbit Monoclonal)	120 µL	4°C
100X Anti- Histone H3 (pSer10) Primary Antibody (Mouse Monoclonal)	120 µL	4°C
1000X AP-Labeled Secondary Antibody (anti-Mouse)	20 µL	4°C
1000X HRP-Labeled Secondary Antibody (anti-Rabbit)	20 µL	4°C
400X Fluorescent Substrate Cocktail	50 µL	4°C
Fluorescent Substrate Buffer	12 mL	4°C
8000X H2O2	50 µL	4°C
10X Quenching Solution	1.5 mL	4°C
Janus Green Stain	17 mL	4°C

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microplate reader capable of measuring fluorescence
- MilliQ water or other type of double distilled water (ddH2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom/96 well plate with clear flat bottom, preferably white/96 well plate with clear flat bottom, preferably black
- Sodium Azide (preservative)
- 0.5 M HCl (optional for Janus Green cell staining procedure).
- Optional humid box for overnight incubation step.
- Optional plate shaker for all incubation steps.

8. TECHNICAL HINTS

- **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 will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

9.1. **1X PBS**

Prepare by diluting 45 mL of 10X PBS in 405 mL of nanopure water or equivalent and mix well. Store at room temperature.

9.2. **1X Wash Buffer**

Prepare by diluting 750 µL of 400X Tween-20 into 300 mL of 1X PBS and mix well. Store at room temperature.

9.3. **8% Paraformaldehyde Solution in PBS.**

Immediately prior to use prepare 8% Paraformaldehyde by combining 6 mL of nanopure water or equivalent, 1.2 mL of 10X PBS and 4.8 mL of 20% Paraformaldehyde.

NOTE*: Paraformaldehyde is toxic and should be prepared and used in a fume hood. Dispose of paraformaldehyde according to local regulations.*

9.4. **1X Quenching Solution**

Prepare by diluting 1.2 mL of 10X Quenching Solution into 10.8 mL of Nanopure water and mix well. Store at room temperature.

9.5. **1X Permeabilization Solution**

Immediately prior to use prepare by diluting 150 µL of 100X Triton X-100 into 15 mL of 1X PBS and mix well.

9.6. **1X Blocking Solution**

Immediately prior to use prepare by diluting 5 mL of 10X Blocking Solution into 45 mL of 1X PBS and mix well.

9.7. **1X Fluorescent Development Solution**

Immediately prior to use prepare by adding 30 µL of the 400X Fluorescent Substrate Cocktail and 1.5 µL of the 8000X H_2O_2 into 12 mL Fluorescent Substrate Buffer and mix well. Discard any excess after completing the experiment.

10. SAMPLE PREPARATION

General Sample Information

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze samples in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- This assay has been optimized for use on adherent cells. For suspension cells, refer to section 15. Ensure that the microplate does not dry out at any time before or during the assay procedure.

10.1. Seed adherent cells directly into an amine coated plate and allow them to attach for >6 hours or overnight. It is advised to seed in a 100 µL volume of the same media used to maintain the cells in bulk culture. The optimal cell-seeding density is cell-type dependent. The goal is to seed cells such that they are just reaching confluency (but not over-confluent) at the time of fixation. As an example, HeLa cells may be seeded at ~ 20,000 cells per well and cultured overnight for fixation the following day.

10.2. The attached cells can be treated if desired with a drug of interest. See Figure 2 of this protocol for suggested positive controls.

10.3. Fix cells by adding a final concentration of 4% Paraformaldehyde Solution. This can be achieved by one of two means: (1) Add a volume of 8% Paraformaldehyde Solution equal to that of the culture volume (e.g. add 100 µL 8% Paraformaldehyde to a well with 100 µL media) *or* (2) gently remove/dump culture media from the wells and replace with 100 µL 4% Paraformaldehyde Solution.

NOTE: *If treatments cause cells to become loosely attached, the overlay fixation option above (1) is recommended. See also FAQ Use of amine coated plates minimizes cell loss, even mitotic cells.*

10.4. Incubate for 10 minutes at room temperature.

10.5. Gently aspirate or dump the Paraformaldehyde Solution from the plate and wash the plate 3 times briefly with 1X PBS. For each wash, rinse each well of the plate with 275 µL of 1X PBS.

10.6. Add 100 µL of 1X PBS with 0.02% sodium azide. The plate can now be stored at 4ºC. Sodium azide will preserve the plate for long storage and it will decrease the peroxidase background normally found on fixed cells.

NOTE: *The plate should not be allowed to dry at any point during or before the assay. Both paraformaldehyde and sodium azide are toxic, handle with care and dispose of according to local regulations.*

11. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to correct temperature prior to use.**
- **We recommended to assay all controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**
- **It is recommended to use a plate shaker (~200 rpm) during all incubation steps. Any step involving removal of buffer or solution should be followed by blotting the plate gently upside down on a paper towel before refilling wells. Unless otherwise noted, incubate at room temperature.**

NOTE: During development of this assay edge effect problems have not been observed. However if edge effects are of concern, the perimeter wells of the plate can be used as control wells (primary antibody omitted). Regardless, one well from which the primary antibodies are excluded to determine background signals of the assay is required.

11.1. Remove the plate with fixed cells from storage and allow it to equilibrate room temperature.

11.2. Remove 1X PBS/Azide (section 10.6) and add 100 µL 1X Quenching Solution to each well of the plate. Incubate 10 minutes at room temperature.

11.3. Wash plate once with 200 µL of 1X PBS solution.

11.4. Remove PBS wash and add 100 µL of 1X Permeabilization Solution to each well of the plate. Incubate for 10 minutes at room temperature.

11.5. Remove Permeabilization Solution and add 200 µL of 1X Block Buffer. Incubate for 2 hours at room temperature.

11.6. Prepare 1X Primary Antibody Cocktail Solution by diluting each of the primary antibodies by 100X into appropriate volume of 1X Blocking Solution (i.e. 12 mL of 1X Blocking Solution + 120 µL of

the 100X Mouse Anti- Histone H3 (pSer10) Primary Antibody + 120 µL of the 100X Rabbit Cdk2 (pTyr15) Primary Antibody).

11.7. Remove 1X Blocking Solution and add 100 µL 1X Primary Antibody Cocktail Solution to each well of the plate. Incubate for 2 hours at room temperature or overnight at 4°C.

NOTE: *To determine the background signal it is essential to omit primary antibody from at least one well containing cells for each experimental condition.*

11.8. Remove 1X Primary Antibody Cocktail Solution and wash the plate 3 times briefly with 1X Wash Buffer. For each wash, rinse each well of the plate with 200 µL of 1X Wash Buffer. **Do not remove the last wash until step 11.10**

11.9. Prepare 1X Secondary Antibody Cocktail Solution by diluting 12 µL of 1000X AP-Labeled Secondary Antibody and 12 µL of 1000X HRP-Labeled Secondary Antibody into of 12 mL 1X Blocking Solution.

11.10. Remove 1X Wash Buffer and add 100 µL 1X Secondary Antibody Cocktail Solution to each well of the plate. Incubate 2 hours at room temperature.

11.11. Remove 1X Secondary Antibody Cocktail Solution and wash 3 times briefly with 1X Wash Buffer. For each wash, rinse each well of the plate with 200 µL of 1X Wash Buffer.

11.12. Wash 2 times with 1X PBS, using 200 µL for each well. **Do not remove the last wash until immediately prior to step 11.13.**

11.13. Remove the 1X PBS and add 100 µL Development Solution to each well of the plate.

11.14. Immediately begin recording in a kinetic mode, or read the plate at a user defined time (e. g. 10 minutes) in an end point mode in the microplate reader prepared with the following settings:

AP Substrate	Excitation wavelength (range)	355 - 365 nm
	Emission wavelength (range)	445 - 455 nm
HRP Substrate	Excitation wavelength (range)	530 - 555 nm
	Emission wavelength (range)	585 - 600 nm
Time	Up to 45 minutes	
Interval	1-5 minutes	
Shaking	Shake between reads	

11.15. Remove the developed solution and wash the plate once with 1X PBS.

11.16. Remove the 1X PBS and add 100 µL of Janus Green Stain to each well of the plate. Incubate plate for 5 minutes at room temperature.

NOTE: *The antibody intensity may be normalized to the Janus Green staining intensity to account for differences in cell seeding density, differential cell growth, or cell loss.*

11.17. Remove the Janus Green Stain and wash the plate 5 times in deionized water or until excess dye is removed.

11.18. Remove last water wash, blot to dry, add 200 µL of 0.5 M HCl to each well of the plate and incubate for 10 minutes in a plate shaker.

11.19. Record the OD at 595 nm using a microplate reader.

12. CALCULATIONS

12.1. For the end point readings, use relative fluorescence units (RFU). For the kinetics readings use change of RFU/unit of time.

12.2. Background subtraction. Determine the fluorescence signal (RFU or change RFU/sec) for the wells that lacked primary antibody (background). Subtract the mean background signal from all other experimental signals.

12.3. Janus Green normalization of both targets. Divide the background-subtracted signals (from 12.2) by the Janus Green OD595 of the corresponding wells. The result is the “normalized intensity”.

13. TYPICAL DATA

Assay performance and specificity were tested using HeLa cells treated with hydroxyurea and paclitaxel. Hydroxyurea inhibits DNA synthesis and arrests cells at the G1/S-phase transition of the cell cycle. Paclitaxel is a microtubule stabilizer and arrests cells at the G2/M phase of the cell cycle.

Figures 1 and 2 show typical results using ab140363. Note that Cdk2 pTyr15 and Histone H3 pSer10 are inversely related to each other as their signal is maximal in different stages of the cell cycle.

Since treatments that induce cell cycle arrest will decrease the relative cell number in treated wells compared to control wells, it is strongly advised to perform the Janus Green cell stain and analyze data according to section 9.2 above.

Antibody Specificity - Because confidence in antibody specificity is critical to ICE data interpretation, the primary antibodies in this kit were validated for specificity by Western blotting (Figure 3).

Reproducibility - ICE results provide accurate quantitative measurements of antibody binding and hence cellular antigen concentrations. The coefficient of the intra-assay of variation of this assay kit for HeLa cells is typically <10% for both analytes. For example, the mean coefficient of the intra-assay of variation of HeLa cells treated

with paclitaxel and hydroxyurea in the experiment described in Figures 1 and 2 was 4.7% for Histone H3 (pSer10) and 5.1% for Cdk2 (pTyr15).

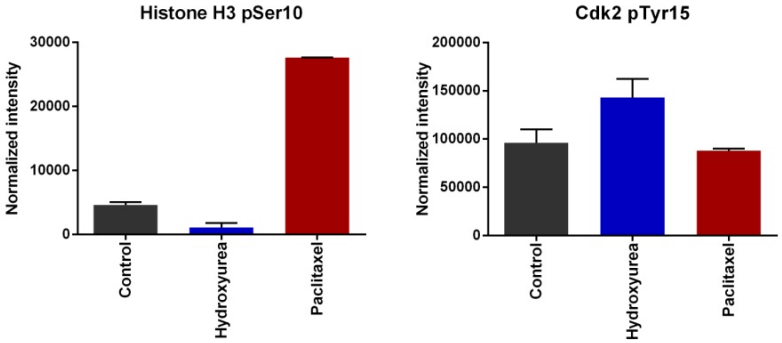


Figure 1. Sample experiment using ab140363 on HeLa cells treated with paclitaxel and hydroxyurea. Data shown is for 24 hour treatment with 1 mM hydroxyurea, 333 nM paclitaxel and untreated (Control). (Normalized intensity is described in section 9.)

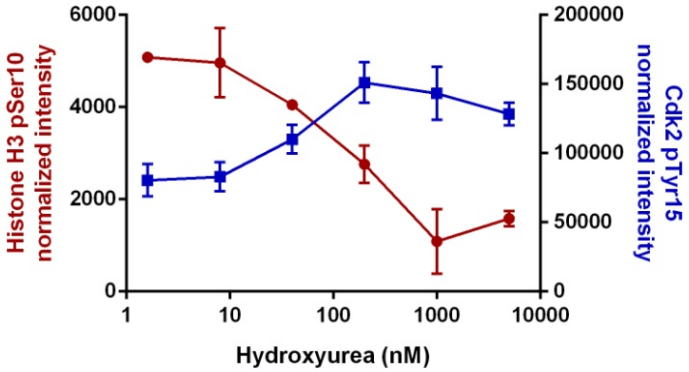


Figure 2. Sample data using ab140363 on HeLa cells treated with a titration of hydroxyurea. Data shown is for 24 hour treatment with 1.6 – 5000 nM Hydroxyurea. Quantification of the data shown in Figure 1. Cdk2 pTyr15 intensity increases with hydroxyurea treatment dose whereas Histone H3 pSer10 intensity decreases.

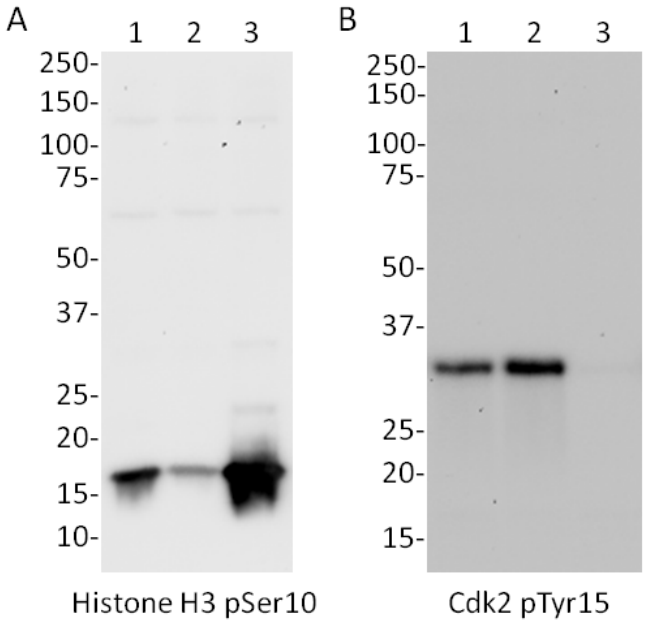


Figure 3. Antibody specificity demonstrated by Western Blot Analysis. Whole cell lysates from HeLa cells were analyzed by western blot with the primary antibodies used in this assay kit. (A) Histone H3 pSer10 antibody: Untreated (lane 1), hydroxyurea = G1/S arrest (lane 2), paclitaxel = G2/M arrest (lane 3). (B) Cdk2 pTyr15 antibody: Untreated (lane 1), thymidine = G1/S arrest (lane 2), nocodazole = G2/M arrest (lane 3).

14. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use

Problem	Cause	Solution
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

15. FAQ

How many cells do I seed per well?

The cell seeding density varies by cell type and depends both on the cell size and the abundance of the target protein. The cell seeding will likely need to be determined experimentally by microscopic density observation of serially diluted cells. For adherent cells, prepare serial dilution of the cells in a plate and allow them to attach prior to observation. The goal is to have cells that are just confluent at the time of fixation. Overly confluent cells may have compromised viability and tend to not adhere as well to the plate. Under-seeded cells may yield too low a signal, depending on the analyte. Keep in mind that drug treatments or culture conditions may affect cell density/growth.

Do I have to use an amine-coated microplate?

We have tested black-wall-amine and cell-culture treated microplates and found that amine coated plates improve reproducibility and specificity in comparison to standard plates. In addition, multiple cell types appear to have the most favorable growth and even seeding on amine plates. The assay performance is only guaranteed with amine plates.

A treatment causes cells detachment. Is there a way to prevent the lost of detaching cells?

Loss of floating cells can be easily prevented by inserting two centrifugation steps into the protocol: (1) Immediately prior the addition of Paraformaldehyde Solution (step 7.3) centrifuge the microtiter plate at 500 xg for 5-10 minutes, (2) Immediately after the addition of Paraformaldehyde Solution centrifuge the microtiter plate again at 500 x g for 5-10 minutes. Continue in the fixation for a total of 15-20 minutes. For examples using detaching cells in ICE, refer to ab110215 Product Booklet.

Can I use suspension cells for ICE?

The In-Cell ELISA can be easily adapted for use with suspension cell. In this case an amine plate must be used. To ensure efficient crosslinking of the suspension cells to the amine plate, cells must be grown and treated in a different plate or dish of choice. The treated suspension cells are then transferred to the amine plate in 100 µL of media per well. The

cell seeding density of the amine plate is cell type-dependent. If necessary, cells can be concentrated by centrifugation and re-suspended in PBS (preferred) or in media to desired concentration. As an example, HL-60 and Jurkat cells should be seeded, respectively, at 300,000 and 200,000 cells per well in 100 µL of PBS (preferred) or media. After the cells are transferred to the amine plate immediately follow the fixation procedure as described in section 11.3. For examples using suspension cells in ICE, refer to ab110215 Product Booklet.

NOTE: *With suspended cells, the media should contain no more than 10 % fetal serum otherwise efficiency of the suspension cell crosslinking to the plate may be compromised.*

I grow my cells in 15% FBS, will this interfere with the cell fixation?

Culture media containing up to 15% fetal serum does not interfere with the cell fixation and cross-linking to the plate.

How do I measure the assay background?

It is essential to omit primary antibody in at least one well (3 wells recommended) to provide a background signal for the experiment which can be subtracted from all measured data. This should be done for each experimental condition.

Can I measure each target in an individual well instead measure both targets simultaneously in a single well?

If the user prefers to use the Cdk2 (pTyr15) antibody and the Histone H3 (pSer10) antibody separately that may be done as well. Sufficient amounts of the secondary antibody cocktail and all buffers are provided to use each of the primary antibodies separately.

Is Janus Green normalization necessary?

Janus Green is a whole-cell stain that is useful to determine if a decrease in signal intensity in a well is due to a relevant down-regulation or degradation of the target analyte OR if it is a function of decreased cell number (e.g. due to cytotoxic effect of a treatment). As such it is not a required readout, but is useful in analysis to determine a normalized intensity value (section 12.2).

What is the chemistry of the development reaction?

Horseradish peroxidase (HRP) converts the supplied substrate (10-acetyl-3,7-dihydroxyphenoxazine, known also as AmplifluRed) in the presence of hydrogen peroxide to highly fluorescent product (resorufin) with excitation maximum 571 nm and emission maximum 585 nm. Alkaline Phosphatase (AP) converts the supplied substrate (4-methylumbelliferyl phosphate, known also as MUP) into a fluorescent molecule with an excitation maximum 360 nm and emission maximum 449 nm. Section 11.14 provides the range of recommend excitation and emission wavelengths. Optimum settings may depend on the specific fluorescence reader used.

16. NOTES

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