Version 1a Last updated 11 June 2020

ab239454 FirePlex® Intracellular Immunoassay Core Reagent Kit Protocol Booklet

For the preparation of cell extracts and tissue homogenates.

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

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1. Overview

Our multiplex immunoassays use the FirePlex® particle technology to quantify up to 70 protein and peptide analytes in the same well, from 12.5 µL sample input.

Assay run-time is 3.5 hours, followed by particle analysis using a validated flow cytometer model and data analysis using our integrated, free-of-charge FirePlex Analysis Workbench software.

The Intracellular Lysis Kit is used for the preparation of cell extracts and tissue homogenate samples for the quantitative measurement of multiple protein targets in a FirePlex immunoassay.

Important - Use of FirePlex Immunoassays requires the purchase of:

a) A FirePlex Immunoassay Core Reagent Kit
b) A FirePlex Immunoassay Panel, such as Human Key Cytokines - Immunoassay Panel
c) A FirePlex Immunoassay Protein Standard Mix such as Human Protein Standard Mix A
d) A FirePlex Intracellular Lysis Kit (ab239454)

2. 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 handled 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.

3. Storage and Stability

Store FirePlex Intracellular Lysis Kit at 4°C immediately upon receipt. Kit has a storage time of 6 months from date of receipt.

Refer to Section 5 for storage conditions of individual components.

4. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

5. Materials Supplied

Item	Quantity	Storage Condition (Before prep)
10X Wash Buffer	25 mL	+4°C
5X Intracellular Extraction Buffer	10 mL	+4°C
50X Intracellular Extraction Enhancer	1 mL	+4°C
2X Intracellular Assay Diluent	15 mL	+4°C

6. Materials Required, Not Supplied

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

- **Protease inhibitors.** We recommend using Protease Inhibitor Cocktail (EDTA-free) (ab201111) at a 1:100 working dilution, or a similar product.
- Test tubes for working stock solutions
- Deionized water
- Vortex mixer
- Microcentrifuge

7. Technical Hints

- The provided 5X Intracellular Extraction Buffer does not contain protease inhibitors. We strongly encourage the addition of protease inhibitors to the 1X Intracellular Extraction Buffer when preparing sample lysates to prevent protein degradation.
- The provided 50X Intracellular Extraction Enhancer Solution may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The 50X Intracellular Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.

8. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment. The instructions below for preparation of 1X Wash Buffer and 1X Intracellular Extraction Buffer.

8.1 1X Wash Buffer

Prepare 1X Wash Buffer by diluting 10X Wash Buffer with deionized water. To prepare 200 mL 1X Wash Buffer, combine 20 mL 10X Wash Buffer with 180 mL deionized water. Mix thoroughly and gently.

8.2 1X Intracellular Extraction Buffer

Prepare 1X Intracellular Extraction Buffer by diluting 5X Intracellular Extraction Buffer and 50X Intracellular Extraction Enhancer Solution with 1X Wash Buffer. To make 20 mL 1X Intracellular Extraction Buffer combine 15.6 mL 1X Wash Buffer, 4 mL 5X Intracellular Extraction Buffer and 0.4 mL 50X Intracellular Extraction Enhancer. Mix thoroughly and gently. Protease inhibitors should be added directly to the 1X Intracellular Extraction Buffer, immediately prior to sample lysis.

8.3 1X Intracellular Lysis Assay Diluent

Prepare according to Section 12 of the FirePlex Immunoassay Panel Protocol Booklet.

9. Sample Preparation

- Recommended sample dilutions for each sample type can be found in Section 14 of the appropriate FirePlex Immunoassay Panel Protocol Booklet. Optimal sample dilutions should however be determined by the end user.
- **For optimal assay performance, samples must always be used either at the recommended dilution or further diluted.**
- **To prevent clogging of the filter plate it is important that samples are clarified via centrifugation as stated below.**
- Samples generating values higher than the highest standard should be further diluted in the 1X Intracellular Lysis Assay Diluent.

9.1 Preparation of extracts from cell pellets:

9.1.1 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.
9.1.2 Rinse cells twice with PBS.
9.1.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Intracellular Extraction Buffer.
9.1.4 Incubate on ice for 20 minutes.
9.1.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.
9.1.6 Transfer the supernatants into clean tubes and discard the pellets.
9.1.7 The sample total protein concentration in the extract should be quantified using a BCA assay or alternative protein assay. Aliquot and store extracts at -80°C until ready for use.
9.1.8 Dilute samples to desired concentration in 1X Intracellular Lysis Assay Diluent and assay samples immediately.

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

9.2.1 Remove growth media and rinse adherent cells 2 times in PBS.
9.2.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer directly to the plate. Use 0.75 mL - 1.5 mL 1X Intracellular Extraction Buffer per confluent 15 cm diameter plate.
9.2.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 20 minutes.
9.2.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

9.2.5 Transfer the supernatants into clean tubes and discard the pellets.
9.2.6 The sample total protein concentration in the extract should be quantified using a BCA assay or alternative protein assay. Aliquot and store extracts at -80°C until ready for use.
9.2.7 Dilute samples to desired concentration in 1X Intracellular Lysis Assay Diluent and assay samples immediately.

9.3 Preparation of extracts from tissue homogenates:

9.3.1 Tissue lysates are typically prepared by homogenization (dounce homogenizer is recommended) of 100 to 200 mg of wet tissue, that is first minced and thoroughly rinsed with PBS. Typically, homogenization is done in 0.5 mL – 1 mL of chilled PBS with protease inhibitors.
9.3.2 For protein extraction, combine 100 µL of tissue homogenate from step 9.3.1 with 0.5 mL – 1 mL of chilled 1X Intracellular Extraction Buffer plus protease inhibitors and mix well. For lower amounts of tissue, adjust volumes accordingly. Aliquot remaining homogenate and store at -80°C for future extraction to avoid repeated freeze/thaw of the homogenate.
9.3.3 Incubate on ice for 20 minutes.
9.3.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.
9.3.5 Transfer the supernatants into clean tubes and discard the pellets.
9.3.6 The sample total protein concentration in the extract should be quantified using a BCA assay or alternative protein assay. Aliquot and store extracts at -80°C until ready for use.
9.3.7 Dilute samples to desired concentration in 1X Intracellular Lysis Assay Diluent and assay samples immediately.

10. Troubleshooting

Problem	Reason	Solution
Precipitate in Enhancer	Precipitation of the Intracellular Extraction Enhancer	Precipitate can be dissolved by gently warming the Intracellular Extraction Enhancer to 37°C.
Difficulty pipetting lysate; viscous lysate	Genomic DNA solubilized	Prepare Intracellular Extraction Buffer without Enhancer. Add the Enhancer to the cell lysate after extraction.
Low protein yield	Low cell counts in sample	Recount cells in sample or reweigh tissue to ensure sufficient sample input prior to extraction
	BCA assay error	Repeat BCA assay quantification
High protein yield	Growth media contamination	Ensure cells are rinsed thoroughly in PBS prior to their harvest for extraction
Low Signal or Sensitivity	Improper storage of the FirePlex protein standards	Store your reconstituted standards at -80°C, all other assay components at +4°C.
	Inadequate reagent volumes or improper dilution	Ensure sufficient incubation times; Check pipettes and ensure correct calibration
Low Particle or Event Count	Particle settling or aggregation	Invert and vortex Capture Particle Solution thoroughly before assay use
	Vacuum too weak	Adjust the vacuum pressure to be ~5 psi
	Incorrect flow cytometer settings	Check for correct Flow Cytometer settings.
Leaky Filter Plate	Failure to blot filter plate wells	Blot the filter plate on dry tissue paper by holding down firmly for 5 seconds
Clogged Filter Plate	High lipid content in biological fluid samples	Centrifuge the samples at 10,000 x g for 10 minutes at 4°C. Re-collect the soluble fraction of the sample.
Uneven buffer removal from wells	Variable particle counts per well	Repeat assay set up. Ensure thorough mixing of 1X Capture Particle Solution before addition to plate

11. Notes

Technical Support

For all FirePlex-related technical support inquiries, please contact FirePlex@abcam.com