

NutriCulture Colorimetric Caspase-8 Activity Assay Kit

Troubleshooting Guide

100 tests

Cat No: C8A100

Use this guide for common signal, sample-preparation, and readout problems in the NutriCulture Colorimetric Caspase-8 Activity Assay Kit. The assay measures cleavage of IETD-pNA and is read at 400 or 405 nm. Sample amount, protein concentration, fresh DTT/PMSF supplementation, light protection, incubation, and blank subtraction are critical parameters specified in the datasheet.

Before You Start - Quick Checks

1. Use at least 1×10^6 cells per sample; the assay procedure uses $1-5 \times 10^6$ cells.
2. Keep protein concentration at 1-4 mg/mL and use 50-200 ug protein per assay.
3. Add DTT immediately before use: 10 mM final in 2x Reaction Buffer and 5 mM final in Lysis Buffer.
4. Add PMSF to Lysis Buffer immediately before use at 1 mM final concentration.
5. Incubate IETD-pNA reactions at 37°C for 1-2 hours protected from light.
6. Read at 400 or 405 nm and subtract background from both induced and uninduced samples.

Problem	Most likely cause	Recommended action
OD value is very low	Cell number or protein concentration may be below the datasheet recommendation.	Use at least 1×10^6 cells, maintain protein concentration at 1-4 mg/mL, and load 50-200 ug protein per assay.
Caspase-8 activity is weak in the induced sample	The induced sample may have insufficient activity or assay reagents may not have been prepared as specified.	Run an uninduced control and an appropriate positive-control condition; the datasheet notes anti-CD95 (Fas) antibody as an example. Verify fresh DTT/PMSF supplementation.
Results are inconsistent between samples	Protein input or lysate preparation may vary between samples.	Measure protein concentration and normalize each assay to 50-200 ug protein in 45 uL Cell Lysis Buffer before adding reaction reagents.
Lysate remains cloudy or contains insoluble material	The clarification step may have been incomplete.	After lysis on ice, centrifuge 10 minutes at 10,000 x g in a cold microcentrifuge and transfer only the supernatant to a fresh tube.
Activity appears to decline during sample handling	Lysates may not have been kept cold or processing may have been delayed.	Use ice-cold Cell Lysis Buffer, incubate samples on ice for 10-30 minutes, clarify in a cold centrifuge, and keep the cytosolic extract on ice until reaction setup.
Reaction Buffer performance is poor	DTT may not have been added immediately before use or may be at the wrong concentration.	Add 10 uL of 1.0 M DTT per 1 mL of 2x Reaction Buffer to obtain 10 mM final DTT immediately before the assay.
Lysis efficiency or activity preservation is poor	The Lysis Buffer may be missing freshly added DTT or PMSF.	Immediately before use, add DTT to 5 mM final and PMSF to 1 mM final as specified.

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Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
High background obscures fold-change calculation	Blank absorbance from lysates and buffers may not have been subtracted.	Subtract the background reading from both induced and uninduced samples before calculating fold increase in Caspase-8 activity.
Signal varies between replicate wells	Replicates or mixing may be inconsistent.	Assay controls and samples at least in duplicate and mix the reaction by pipetting up and down after adding Reaction Buffer and IETD-pNA.
Signal is weaker than expected after substrate addition	Incubation time, temperature, or light exposure may not match the protocol.	Incubate at 37°C for 1-2 hours protected from light after adding 5 uL IETD-pNA.
Absorbance was measured at the wrong wavelength	The plate reader may not be configured for the pNA readout specified in the datasheet.	Measure absorbance at 400 or 405 nm.
IETD-pNA performance declines during storage	The substrate may have undergone repeated freeze-thaw cycles or excessive light exposure.	Aliquot IETD-pNA into smaller quantities, store at -20°C in the dark, and avoid repeated freezing and thawing.
Cells were harvested outside the recommended density range	The culture may not have been at the stated 70-80% density before induction and collection.	Standardize cultures at 70-80% density before induction and keep seeding/growth conditions consistent between induced and control groups.
Incorrect final reaction volume or component sequence	The sample may not have been normalized before adding reaction reagents.	Adjust 50-200 ug protein to 45 uL Lysis Buffer, add 50 uL 2x Reaction Buffer, then add 5 uL IETD-pNA as specified.

Still having trouble? Recheck cell number, protein concentration, fresh DTT/PMSF supplementation, lysate clarification, 37°C light-protected substrate incubation, and 400/405 nm reading. Background must be subtracted from both induced and uninduced samples before calculating fold increase.