

# Application Guide

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## Empiria Studio® Software Sample Images Guide



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## Table of Contents

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	<b>Page</b>
<b>I. Validate</b> .....	<b>2</b>
Antibody Validation .....	2
Add antibody details page .....	3
Add lane details page .....	3
Total Protein Stain and Target Linear Range Determination .....	4
Housekeeping Protein and Target Linear Range Determination .....	5
Housekeeping Protein Validation .....	6
<b>II. Target Analysis</b> .....	<b>6</b>
Analysis: Target and Total Protein Stain .....	6
Analysis: Target and Housekeeping Protein .....	8

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## I. Validate

### Antibody Validation

This is sample data from an experiment to evaluate the specificity and selectivity of a primary antibody.

## Add antibody details page

	Primary Antibody	Secondary Antibody
Name	Cleaved PARP (Asp214) Antibody (Human Specific)	IRDye® 800CW Goat anti-Rabbit IgG (H + L), Highly Cross-Adsorbed
Vendor	Cell Signaling Technology	LI-COR Biosciences
Product #	5625S	925-3211
Lot #	XYZ	XYZ
Dilution	1:1,000	1:15,000
Incubation Time	1 hour	1 hour
Species	Rabbit	Goat
Diluent	Odyssey® Blocking Buffer (TBS) + .2% Tween 20	Odyssey® Blocking Buffer (TBS) + .2% Tween 20
Immunogen	Asp214	
Notes	MW (kDa): 89	None

## Add lane details page

Lane	Amount (µg)	Description
Lane 01	NA	Chameleon® Pre-stained Protein Ladder (800) MW (kDa): 260, 125, 70, 38, 25, 8
Lane 02	10	Jurkat cell lysate
Lane 03	10	Jurkat cell lysate
Lane 04	10	Jurkat cell lysate
Lane 05	5	Jurkat cell lysate
Lane 06	5	Jurkat cell lysate
Lane 07	5	Jurkat cell lysate

## Total Protein Stain and Target Linear Range Determination

This is a 2-fold dilution series of Jurkat cell lysate to determine the linear response range of total protein (used for normalization in experimental samples) and the target protein.

- Revert™ 700 Total Protein Stain, 700 nm channel (red)
- GAPDH, 800 nm channel (green)

Lane	Amount (µg)	Description
Lane 01	NA	Chameleon Duo Pre-stained Protein Ladder  700 Channel (red) MW (kDa): 160, 90, 50, 30, 15, 8  800 Channel (green) MW (kDa): 260, 125, 70, 38, 25, 8
Lane 02	40	Jurkat cell lysate
Lane 03	20	Jurkat cell lysate
Lane 04	10	Jurkat cell lysate
Lane 05	5	Jurkat cell lysate
Lane 06	2.5	Jurkat cell lysate
Lane 07	1.25	Jurkat cell lysate
Lane 08	0.625	Jurkat cell lysate
Lane 09	0.313	Jurkat cell lysate
Lane 10	0.156	Jurkat cell lysate

## Housekeeping Protein and Target Linear Range Determination

This is a 2-fold dilution series of Jurkat cell lysate to determine the linear response range of a housekeeping protein (used for normalization in experimental samples) and the target protein.

- Housekeeping Protein (COXIV) 17 kDa, 700 channel (red)
- Target: Bcl2 28 kDa, 800 channel (green)

Lane	Amount (µg)	Description
Lane 01	NA	Chameleon Duo Pre-stained Protein Ladder 700 Channel (red) MW (kDa): 160, 90, 50, 30, 15, 8 800 Channel (green) MW (kDa): 260, 125, 70, 38, 25, 8
Lane 02	40	Jurkat cell lysate
Lane 03	20	Jurkat cell lysate
Lane 04	10	Jurkat cell lysate
Lane 05	5	Jurkat cell lysate
Lane 06	2.5	Jurkat cell lysate
Lane 07	1.25	Jurkat cell lysate
Lane 08	0.625	Jurkat cell lysate
Lane 09	0.313	Jurkat cell lysate
Lane 10	0.156	Jurkat cell lysate

## Housekeeping Protein Validation

Determine if the expression of a housekeeping protein (tubulin) was affected by treatment conditions. Jurkat cells were treated with etoposide to induce apoptosis. Cell lysate samples were loaded in triplicate. Total protein staining was performed to confirm transfer efficiency and uniform sample loading.

- Revert 700 Total Protein Stain, 700 nm channel (red)
- Housekeeping protein: ( $\alpha$ -tubulin mouse monoclonal antibody) 52 kDa, 800 nm channel (green)

Lane	Concentration	Description	Comments
Lane 01	NA	Chameleon Duo Pre-stained Protein Ladder 700 Channel (red) MW (kDa): 160, 90, 50, 30, 15, 8 800 Channel (green) MW (kDa): 260, 125, 70, 38, 25, 8	
Lane 02	0%	Jurkat cell lysate (5 $\mu$ g), untreated control	Technical Replicate
Lane 03	0%	Jurkat cell lysate (5 $\mu$ g), untreated control	Technical Replicate
Lane 04	0%	Jurkat cell lysate (5 $\mu$ g), untreated control	Technical Replicate
Lane 05	33%	Jurkat cell lysate (5 $\mu$ g), treated with 33% etoposide	Technical Replicate
Lane 06	33%	Jurkat cell lysate (5 $\mu$ g), treated with 33% etoposide	Technical Replicate
Lane 07	33%	Jurkat cell lysate (5 $\mu$ g), treated with 33% etoposide	Technical Replicate
Lane 08	66%	Jurkat cell lysate (5 $\mu$ g), treated with 66% etoposide	Technical Replicate
Lane 09	66%	Jurkat cell lysate (5 $\mu$ g), treated with 66% etoposide	Technical Replicate
Lane 10	66%	Jurkat cell lysate (5 $\mu$ g), treated with 66% etoposide	Technical Replicate
Lane 11	99%	Jurkat cell lysate (5 $\mu$ g), treated with 99% etoposide	Technical Replicate
Lane 12	99%	Jurkat cell lysate (5 $\mu$ g), treated with 99% etoposide	Technical Replicate
Lane 13	99%	Jurkat cell lysate (5 $\mu$ g), treated with 99% etoposide	Technical Replicate

## II. Target Analysis

### Analysis: Target and Total Protein Stain

Quantitative Western blot analysis of Bcl-2 expression in Jurkat cells treated with increasing concentrations of etoposide and normalized using Revert 700 Total Protein Stain.

- Revert 700 Total Protein Stain 700 nm channel (red)
- Target: Bcl2 28 kDa, 800 nm channel (green)

Lane	Concentration	Description	Comments
Lane 01	NA	Chameleon Duo Pre-stained Protein Ladder  700 Channel (red) MW (kDa): 160, 90, 50, 30, 15, 8  800 Channel (green) MW (kDa): 260, 125, 70, 38, 25, 8	
Lane 02	0%	Jurkat cell lysate (5 µg), untreated control	Technical Replicate
Lane 03	0%	Jurkat cell lysate (5 µg), untreated control	Technical Replicate
Lane 04	0%	Jurkat cell lysate (5 µg), untreated control	Technical Replicate
Lane 05	33%	Jurkat cell lysate (5 µg), treated with 33% etoposide	Technical Replicate
Lane 06	33%	Jurkat cell lysate (5 µg), treated with 33% etoposide	Technical Replicate
Lane 07	33%	Jurkat cell lysate (5 µg), treated with 33% etoposide	Technical Replicate
Lane 08	66%	Jurkat cell lysate (5 µg), treated with 66% etoposide	Technical Replicate
Lane 09	66%	Jurkat cell lysate (5 µg), treated with 66% etoposide	Technical Replicate
Lane 10	66%	Jurkat cell lysate (5 µg), treated with 66% etoposide	Technical Replicate
Lane 11	99%	Jurkat cell lysate (5 µg), treated with 99% etoposide	Technical Replicate
Lane 12	99%	Jurkat cell lysate (5 µg), treated with 99% etoposide	Technical Replicate
Lane 13	99%	Jurkat cell lysate (5 µg), treated with 99% etoposide	Technical Replicate

## Analysis: Target and Housekeeping Protein

Quantitative Western blot analysis of Bcl-2 expression in Jurkat cells treated with increasing concentrations of etoposide and normalized using the housekeeping protein tubulin.

- Housekeeping Protein ( $\alpha$ -tubulin mouse monoclonal antibody) 52 kDa, 700 nm channel (red)
- Target: Bcl2 28 kDa, 800 channel (green)

Lane	Concentration	Description	Comments
Lane 01	NA	Chameleon Duo Pre-stained Protein Ladder  700 Channel (red) MW (kDa): 160, 90, 50, 30, 15, 8  800 Channel (green) MW (kDa): 260, 125, 70, 38, 25, 8	
Lane 02	0%	Jurkat cell lysate (5 $\mu$ g), untreated control	Technical Replicate
Lane 03	0%	Jurkat cell lysate (5 $\mu$ g), untreated control	Technical Replicate
Lane 04	0%	Jurkat cell lysate (5 $\mu$ g), untreated control	Technical Replicate
Lane 05	33%	Jurkat cell lysate (5 $\mu$ g), treated with 33% etoposide	Technical Replicate
Lane 06	33%	Jurkat cell lysate (5 $\mu$ g), treated with 33% etoposide	Technical Replicate
Lane 07	33%	Jurkat cell lysate (5 $\mu$ g), treated with 33% etoposide	Technical Replicate
Lane 08	66%	Jurkat cell lysate (5 $\mu$ g), treated with 66% etoposide	Technical Replicate
Lane 09	66%	Jurkat cell lysate (5 $\mu$ g), treated with 66% etoposide	Technical Replicate
Lane 10	66%	Jurkat cell lysate (5 $\mu$ g), treated with 66% etoposide	Technical Replicate
Lane 11	99%	Jurkat cell lysate (5 $\mu$ g), treated with 99% etoposide	Technical Replicate
Lane 12	99%	Jurkat cell lysate (5 $\mu$ g), treated with 99% etoposide	Technical Replicate
Lane 13	99%	Jurkat cell lysate (5 $\mu$ g), treated with 99% etoposide	Technical Replicate





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