






Bio-Rad's Host Cell Protein (HCP) Workflow

Gold Standard for Regulatory Compliance

Qualify HCP ELISA antibodies by 2-D electrophoresis and western blotting in less than 2 days.

Workflow Step	Products	Benefit
1 Sample preparation 	ReadyPrep™ 2-D Cleanup Kit	<ul style="list-style-type: none"> Removes ionic contaminants such as detergents and lipids Concentrates dilute samples Improves 2-D resolution and reproducibility
	ReadyPrep 2-D Starter Kit	<ul style="list-style-type: none"> Includes all components for strip rehydration, focusing, and equilibration prior to second dimension
2 2-D electrophoresis 	ReadyStrip™ IPG Strips	<ul style="list-style-type: none"> For first-dimension separation using immobilized pH gradients Available in different pH ranges
	PROTEAN® i12™ IEF System	<ul style="list-style-type: none"> For first-dimension electrophoresis with individual lane control
	Criterion™ Dodeca™ Cell Criterion™ TGX™ Precast Gels Precision Plus Protein™ Standard Plugs	<ul style="list-style-type: none"> For second-dimension separation by molecular mass
	SYPRO Ruby Protein Gel Stain or Oriole™ Fluorescent Gel Stain  Replicate gel stained with Oriole Stain.	<ul style="list-style-type: none"> For gel evaluation. No interference with subsequent blotting and analysis
	ChemiDoc™ MP or ChemiDoc Imaging System	<ul style="list-style-type: none"> For imaging and analysis
3 Protein transfer 	Trans-Blot® Turbo™ Transfer Starter System Trans-Blot Turbo RTA Midi LF PVDF Transfer Kit	<ul style="list-style-type: none"> For rapid high-efficiency transfer to PVDF membranes
4 Imaging 	Clarity™ Western ECL Substrate Clarity Max™ Western ECL Substrate	<ul style="list-style-type: none"> Femtogram-level detection of proteins with HRP-conjugated secondary antibodies with 24 hour signal duration
	SYPRO Ruby Protein Blot Stain	<ul style="list-style-type: none"> For total protein blot staining
	ChemiDoc MP or ChemiDoc Imaging System	<ul style="list-style-type: none"> For imaging and analysis
5 Analysis software 	PDQuest™ 2-D Analysis Software  Total HCP stained blot. Chemiluminescent anti-HCP western blot. * The SameSpots Software is also recommended for 2-D analysis.	<ul style="list-style-type: none"> Allows automated spot detection and matching PDQuest is compatible up to OS Windows XP <p>SameSpots Software:</p> <ul style="list-style-type: none"> Compatible with OS Windows 7 or higher Equivalent features to the PDQuest 2-D Analysis Software

FAQs

Q1: Why should I consider 2-D electrophoresis (2-DE) and western blotting to evaluate antibodies used in HCP ELISA/immunoassay?

For adequate performance of the ELISA/immunoassay, ICH Q6B guidelines specify that the polyclonal antibodies used must be capable of detecting a wide range of protein impurities. The U.S. Food and Drug Administration (FDA) recommends that the antiserum should be qualified for its ability to detect potential HCP impurities and that this qualification/evaluation be done via 2-DE and western blotting. Per FDA guidance, 1-DE and western blotting is not sufficiently informative for this purpose, and other methods may be used in consultation with the agency, if they are as sensitive and discriminating as 2-DE and western blotting.

Q2: What is the recommended match rate (percentage of HCPs detectable by a sensitive protein stain and immunoreactive against the anti-HCP antiserum/antibodies) by 2-DE and western blotting?

There is no regulatory recommendation on this, but the assumption is that the higher the match rate, the better the

anti-HCP antiserum. Some researchers require a match rate of ~80% before qualifying the antibodies for use in HCP ELISA. Lower match rates may be acceptable if it can be demonstrated that the nondetectable impurities do not copurify with the final product or they are monitored by orthogonal methods.

Bio-Rad has developed a dependable 2-DE and western blotting solution for highly consistent, accelerated antibody evaluations in less than 2 days that can help guide downstream decisions for effective HCP monitoring.

What resources are available?

Berkelman T et al. (2013). Enhanced 2-D electrophoresis and western blotting workflow for reliable evaluations of anti-HCP antibodies. *Bioprocess Int* 11, 50–61.

Berkelman T et al. (2013). Reliable, streamlined 2-D western blot workflow for evaluation of antibodies developed for detection of host cell proteins. *Bio-Rad Bulletin* 6393.

Rusbult JJ et al. (2013). Effectiveness of 2-D electrophoresis over 1-D electrophoresis followed by western blotting for evaluating strategies used to generate host cell protein antibodies. *Bio-Rad Bulletin* 6405.

2-D electrophoresis workflow: How-to-guide. *Bio-Rad Bulletin* 2651.

Ordering Information

Catalog # Description

Protein Sample Preparation

1632130 **ReadyPrep 2-D Cleanup Kit for IEF**, 50 preps
1632105 **ReadyPrep 2-D Starter Kit**, includes protein sample and reagents sufficient to rehydrate, focus, and transfer to second-dimension gels for six 17 cm, ten 11 cm, or sixteen 7 cm ReadyStrip IPG Strips

2-D Electrophoresis Instrumentation

1646000 **PROTEAN i12 IEF System**, 90–240 VAC, includes basic unit and accessories to accommodate 7, 11, and 17 cm IPG strips

ReadyStrip IPG Strips (12 per package)

	7 cm	11 cm	17 cm	18 cm	24 cm
pH 3–10	1632000	1632014	1632007	1632032	1632042
pH 3–10 NL	1632002	1632016	1632009	1632033	1632043
pH 3–6	1632003	1632017	1632010	1632035	1632045
pH 4–7	1632001	1632015	1632008	1632034	1632044
pH 5–8	1632004	1632018	1632011	1632036	1632046
pH 7–10	1632005	1632019	1632012	1632037	1632047

NL, nonlinear.

Electrophoresis System

1656001 **Criterion Cell**, vertical midi-format electrophoresis cell, includes buffer tank, lid with power cables, 3 sample loading guides (12+2-well, 18-well, 26-well)

Criterion TGX Precast Midi Protein Gels (for 11 cm IPG Strips)

5671071 **18% Criterion TGX Precast Gel**
5671081 **4–15% Criterion TGX Precast Gel**
5671091 **4–20% Criterion TGX Precast Gel**
5671101 **8–16% Criterion TGX Precast Gel**
5671111 **10–20% Criterion TGX Precast Gel**
5671121 **Any kD™ Criterion TGX Precast Gel**

Protein Standards

1610378 **Precision Plus Protein Standard Plugs**, pkg of 24, 1 mm thick agarose plugs containing 10 *Strep*-tagged recombinant proteins (10–250 kD), including 3 reference bands

Total Protein Gel Stain

1610496 **Oriole Fluorescent Gel Stain**, 1x solution, 1 L
1703125 **SYPRO Ruby Protein Gel Stain**, 1x solution, 1 L

Catalog # Description

Blotting System

1704155 **Trans-Blot Turbo Transfer Starter System**, blotting instrument, includes base, 2 cassettes to hold 1–2 midi or up to 4 mini blotting sandwiches, blot roller, and starter consumables kit
1704275 **Trans-Blot Turbo RTA Midi LF PVDF Transfer Kit**, for 40 blots

Total Protein Blot Stain

1703127 **SYPRO Ruby Protein Blot Stain**, 200 ml

Detection Reagents

1705060 **Clarity Western ECL Substrate**, 200 ml
1705061 **Clarity Western ECL Substrate**, 500 ml
1705062 **Clarity Max Western ECL Substrate**, 100 ml

Imaging Systems

17001402 **ChemiDoc MP Imaging System**, includes internal computer, 12" touch-screen display, camera, Image Lab™ Touch Software, blot/UV/stain-free sample tray, Image Lab Desktop Software, 200 ml Clarity Substrate, 100 ml Clarity Max Substrate, 500 µl Precision Plus Protein All Blue Prestained Standards, 1 ml Precision Plus Protein Unstained Standards
17001401 **ChemiDoc Imaging System**, includes internal computer, 12" touch-screen display, camera, Image Lab Touch Software, blot/UV/stain-free sample tray, Image Lab Desktop Software, 200 ml Clarity Substrate, 100 ml Clarity Max Substrate, 500 µl Precision Plus Protein All Blue Prestained Standards, 1 ml Precision Plus Protein Unstained Standards

Analysis Software

1709630 **PDQuest Advanced 2-D Analysis Software**

* SameSpots 2-D Analysis Software (Totalab.com/samespots).

Visit bio-rad.com/web/2-D/HCPWorkflow for more information.

SameSpots is a trademark of TotalLab Limited. SYPRO is a trademark of Life Technologies Corporation. Bio-Rad Laboratories, Inc. is licensed by Life Technologies Corporation to sell SYPRO products for research use only under U.S. Patent Number 5,616,502.

Clarity Max Western ECL Substrate is manufactured by Cyanagen Sri and is the subject of patent application numbers US7855287, EP1950207, US9040252, AU2011202658, CA2742025, US8129136, and EP1962095, together with other equivalent granted patents and patent applications in other countries like CN102313732.

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