PiQ™ ECL Substrate Kit #636

GenuIN BIOTECH
Real Matters

Catalog & Pricing

Cat. #	Description	Size
636-50	Use with HRP in chemiluminescent ELISA	50 mL
636-100		100 mL
636-200		200 mL

Background

Enhanced chemiluminescence (ECL) substrates are used with horseradish peroxidase (HRP)-conjugated secondary antibodies to detect protein abundance in immunoassays. HRP catalyzes luminol oxidation in the presence of hydrogen peroxide, producing light that can be detected by an X-ray film, digital imager or chemiluminescence plate reader.

PiQ™ ECL Substrate Kit is formulated with proprietary enhancers that significantly increase the intensity and duration of light emitted. The PiQ™ ECL reagents are ultrasensitive and suitable for detecting proteins at the picogram level. When using these reagents in Western blotting, the recommended dilutions for primary and secondary antibodies are 1:5,000 and 1:50,000, respectively. We also tested this product in chemiluminescent ELISA, it outperformed other commercially available ECL reagents by generating much stronger signals with a relative low background.

Product Features

- Superior performance in generating extremely intense signals
- Suitable for detecting low-abundance analytes
- Optimized for Western blotting and chemiluminescent ELISA

Storage

Stable at 4°C for at least 1 year.

Kit Component

Equal volume of Reagents A and B

Experimental Protocol

1. Let the ECL reagents warm up at room temperature for approximately 30 min prior to use.
2. Combine Reagents A and B at a ratio of 1:1. Vortex to mix.
3. Apply the mixture to the Western blots or microplate wells.
4. Immediately capture signals with a digital imager or chemiluminescence plate reader.

APPLICATION TESTED

Western Blotting
Chemiluminescent ELISA

Validation Data

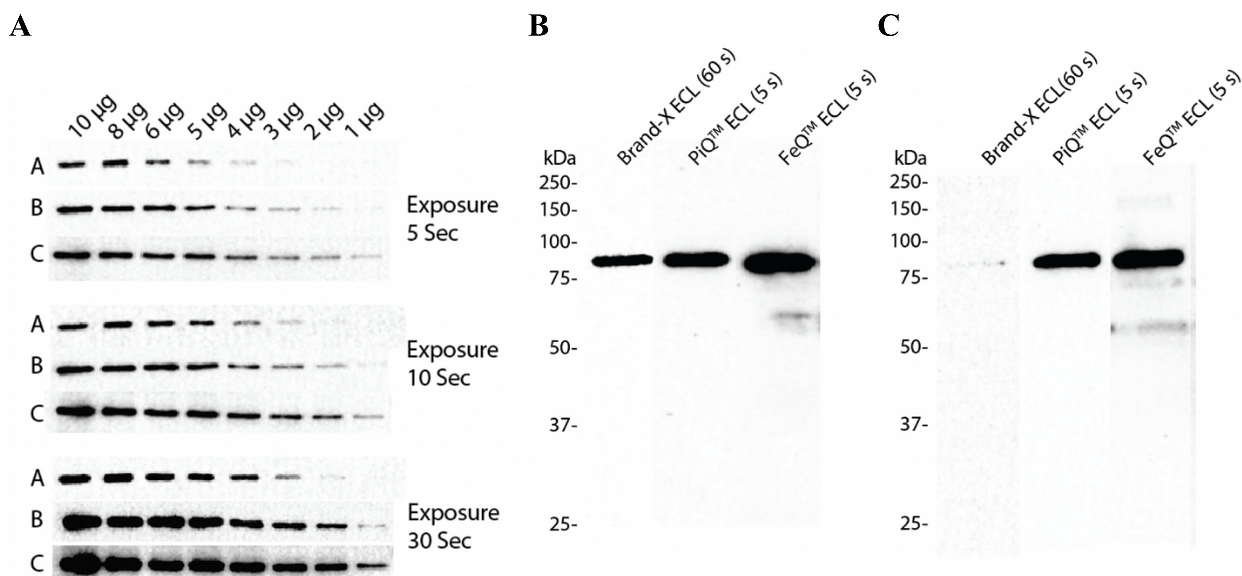

Figure 1. Comparison of the sensitivity of PiQ™, FeQ™, and a commercial ECL kit (Brand-X). (**A**) HEK293 cell lysates were separated by SDS-PAGE and blotted with an anti-ACTB antibody (Cat# 1171, 1:2,000) and an HRP-conjugated goat anti rabbit IgG secondary antibody (Cat# 202, 1:2,000). Thirty micrograms of HeLa (**B,** human) and DF-1 (**C,** chicken) cell lysates were blotted with an anti-RRM1 (Cat#9651, 1:1,000) antibody and an HRP-conjugated goat anti rabbit IgG secondary antibody (Cat# 202, 1:2,000). **Note**: Due to weak cross-reactivity of the antibody with chicken RRM1, brand-X ECL failed to pick up the band on the Western blot, yet the PiQ™ and FeQ™ ECL can detect the protein with a strong band.

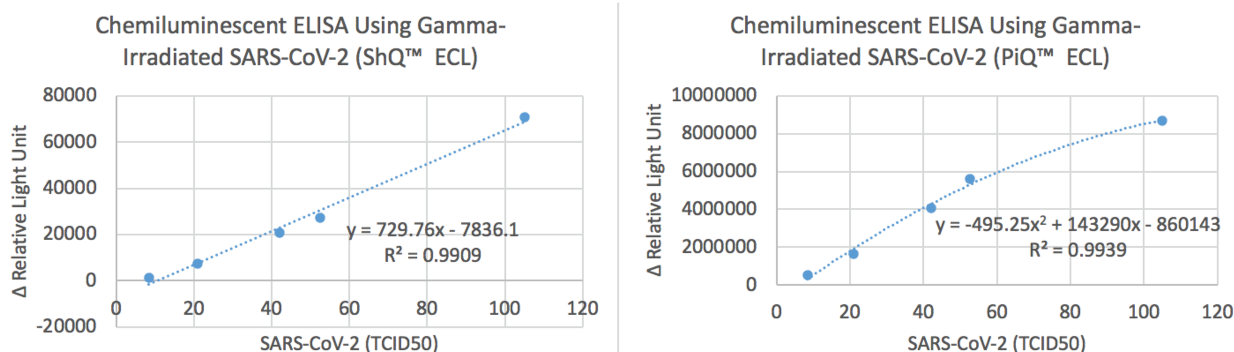

Figure 2. Comparison of ShQ™ ECL and PiQ™ ECL sensitivity in chemiluminescent ELISA. Different concentrations of gamma-irradiated SARS-CoV-2 viruses were lysed in the Viral Lysis Buffer (Cat# VL101) and coated (150 µL) on microplate wells. A rabbit monoclonal anti-N primary (Cat# VYN7, 1:10,000) and an HRP-conjugated highly cross-adsorbed goat anti rabbit IgG secondary antibody (Cat# 202, 1:20,000) were used to detect SARS-CoV-2 N protein. ShQ™ ECL (Cat# 707) and PiQ™ ECL (Cat# 636) Substrate Kits were used to generate chemiluminescent signals. RLU, relative light unit. ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal. **Note**: When the antigen is abundant, ShQ™ ECL is a better choice based on linear regression data.

Table 1. Comparison of ShQ™ ECL and PiQ™ ECL sensitivity and background noise.

	ShQ™ ECL			PiQ™ ECL		
	w/o 1st Ab	w/ 1st Ab	Δ RLU	w/o 1st Ab	w/ 1st Ab	Δ RLU
Lysis buffer	3056	2927	-129	130727	137531	6804
Lysis buffer w/o 2nd Ab	2860	2607	-253	122382	127826	5444
105 TCID50 SARS-CoV-2	2781	73695	70914	128401	8817960	8689559
52.5 TCID50 SARS-CoV-2	2987	30316	27329	134264	5748869	5614605
42 TCID50 SARS-CoV-2	2726	23527	20801	128083	4198178	4070095
21 TCID50 SARS-CoV-2	2767	10342	7575	131388	1797070	1665682
8.4 TCID50 SARS-CoV-2	2901	4143	1242	129277	635622	506345

Notes:

1. Different concentrations of gamma-irradiated SARS-CoV-2 viruses were lysed in the Viral Lysis Buffer (Cat# VL101) and coated (150 µL) on microplate wells.
2. A rabbit monoclonal anti-N antibody (Cat# VYN7, 1:10,000) and an HRP-conjugated highly cross-adsorbed goat anti rabbit IgG secondary antibody (Cat# 202, 1:20,000) were used to detect SARS-CoV-2 N protein.
3. ShQ™ ECL (Cat# 707) and PiQ™ ECL (Cat# 636) Substrate Kits were used to generate chemiluminescent signals.
4. RLU, relative light unit. ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.