abcam

Application note

Faster ELISA results with CatchPoint® SimpleStep ELISA® kits

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Introduction

A conventional sandwich ELISA uses a pair of antibodies against the target protein, one capture antibody coated on the wells of a microplate, and one detector antibody usually conjugated to enzymes such as horseradish peroxidase (HRP) or alkaline phosphatase (AP). Depending on the substrate used for these enzymes, the result can be measured on a microplate reader as an absorbance or fluorescence signal.

A typical ELISA protocol is time-consuming, with multiple incubation and wash steps. Abcam's SimpleStep ELISA® technology drastically reduces assay time by forming and capturing the antibody-analyte sandwich complex in the well of a microplate precoated with an immunoaffinity tag in a single step. The protocol requires only one incubation and one wash step before the signal is developed, requiring just 90 minutes (Figure 1).

CatchPoint® SimpleStep ELISA® uses the CatchPoint dye, Stoplight Red, to provide a fluorescence-based detection. The fluorescent assay offers a broader dynamic range with increased upper and lower limits of quantification. The fluorescent signal is also more stable, allowing detection anywhere from 10 minutes to at least one hour after substrate addition.

Benefits

- Reduce time to results by nearly two-thirds compared to conventional ELISA with a single-wash, 90-minute protocol
- Increase assay signal and dynamic range with fluorescent readout
- Generate standard curves and quantitate results quickly with a preconfigured protocol in SoftMax Pro Software

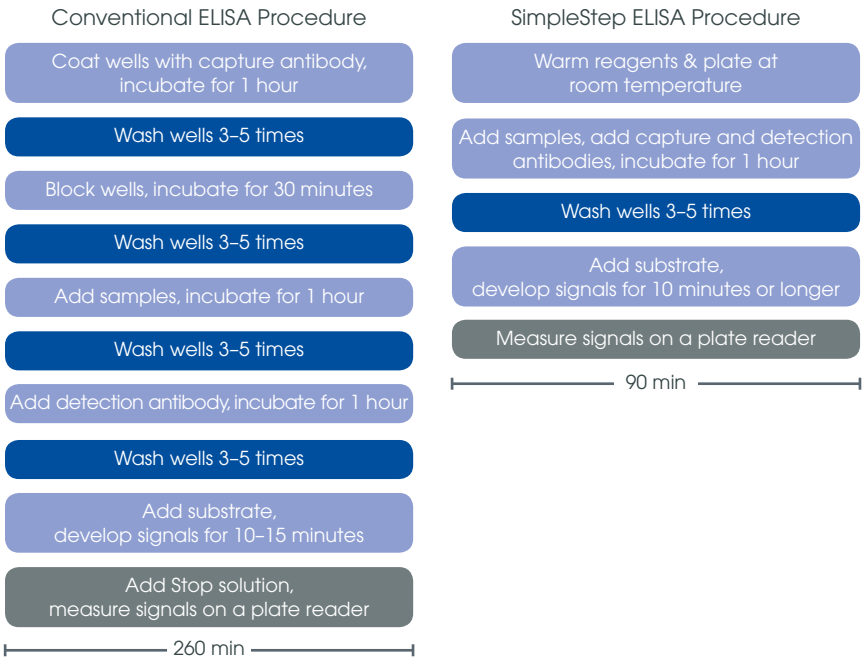

Figure 1.Workflow comparison of conventional ELISA and SimpleStep ELISA protocols. SimpleStep ELISA technology reduces protocol time by nearly two thirds.

Materials

CatchPoint SimpleStep ELISA kits
– Human Pro-Collagen I alpha 1 ELISA Kit, Fluorescent (Abcam, ab229389)
– Human Apolipoprotein A-IV ELISA Kit, Fluorescent (Abcam, ab229428)
– Human TNF alpha ELISA Kit, Fluorescent (Abcam, ab229399)
– Mouse Heme Oxygenase 1 ELISA Kit, Fluorescent (Abcam, ab229431)
– Mouse MCP3 ELISA Kit, Fluorescent (Abcam, ab229391)
– Mouse Pro-Collagen I alpha 1 ELISA Kit, Fluorescent (Abcam, ab229425)

SimpleStep ELISA kits
– Human Pro-Collagen I alpha 1 ELISA Kit (Abcam, ab210966)
– Human Apolipoprotein A-IV ELISA Kit (Abcam, ab214567)
– Human TNF alpha ELISA Kit (Abcam, ab181421)
– Mouse Heme Oxygenase 1 ELISA Kit (Abcam, ab204524)
– Mouse MCP3 ELISA Kit (Abcam, ab205571)
– Mouse Pro-Collagen I alpha 1 ELISA Kit (Abcam, ab210579)

Molecular Devices microplate readers
– SpectraMax® iD5 Multi-Mode Microplate Reader (cat. #ID5-STD)
– SpectraMax® i3x Multi-Mode Microplate Reader (cat. #i3X)
– SpectraMax® M4 and M5 Multi-Mode Microplate Readers (cat. #M4 or M5)
– FilterMax™ F5 Multi-Mode Microplate Reader (cat. #F5)

Methods

Standard curves were generated following the assay protocols provided with each kit. The colorimetric signal development was stopped after 10 minutes. The fluorescent signals were measured at 10 minutes and one hour after the Stoplight Red substrate was added, without the need for adding a stop solution.

Fluorescent signals from the same assay plate were measured on the SpectraMax iD5 reader (the SpectraMax iD3 reader is also suitable for these assays, as it has the same onboard fluorescence performance as the SpectraMax iD5 reader), SpectraMax i3x, SpectraMax M4 or M5, and FilterMax F5 Multi-Mode Microplate Readers. The SpectraMax M4 reader had identical performance specifications to the SpectraMax M5 reader for absorbance and fluorescence detection. Preconfigured SoftMax® Pro Software protocols (available at softmaxpro.org) with optimized instrument settings (Table 1) were used to generate and analyze data.

Parameter	SpectraMax iD5	SpectraMax i3x	SpectraMax M4/M5	FilterMax F5
Optical configuration	N/A	Monochromator	N/A	N/A
Read mode	FL (fluorescence)			
Read type	Endpoint			
Wavelengths	Ex: 530 Em: 590 Do not select 'Use Filter'	Ex: 530 Em: 590	Ex: 530 Em: 590 (Cutoff: 570)	Ex: 535 Em: 595
Plate types	96 w standard opaque			
PMT and optics	PMT Gain: Automatic Integration time: 500 ms Read from top Read height: 0.78 mm	PMT Gain: High Flashes per read: 10 Read from top Read height: 0.8 mm	PMT Gain: Automatic Flashes per read: 10 Read from top	Integration time: 200 ms Read from top Read height: 0.5 mm

Table 1. SpectraMax reader settings for CatchPoint SimpleStep ELISA detection. Both SpectraMax iD5 and SpectraMax M5 readers use monochromator-based optics, so there is no need to select an optical configuration in the settings. The SpectraMax i3x reader can use monochromators or detection cartridges containing filters (monochromators are sufficient for CatchPoint SimpleStep ELISA detection). The FilterMax F5 reader only uses filters for detection.

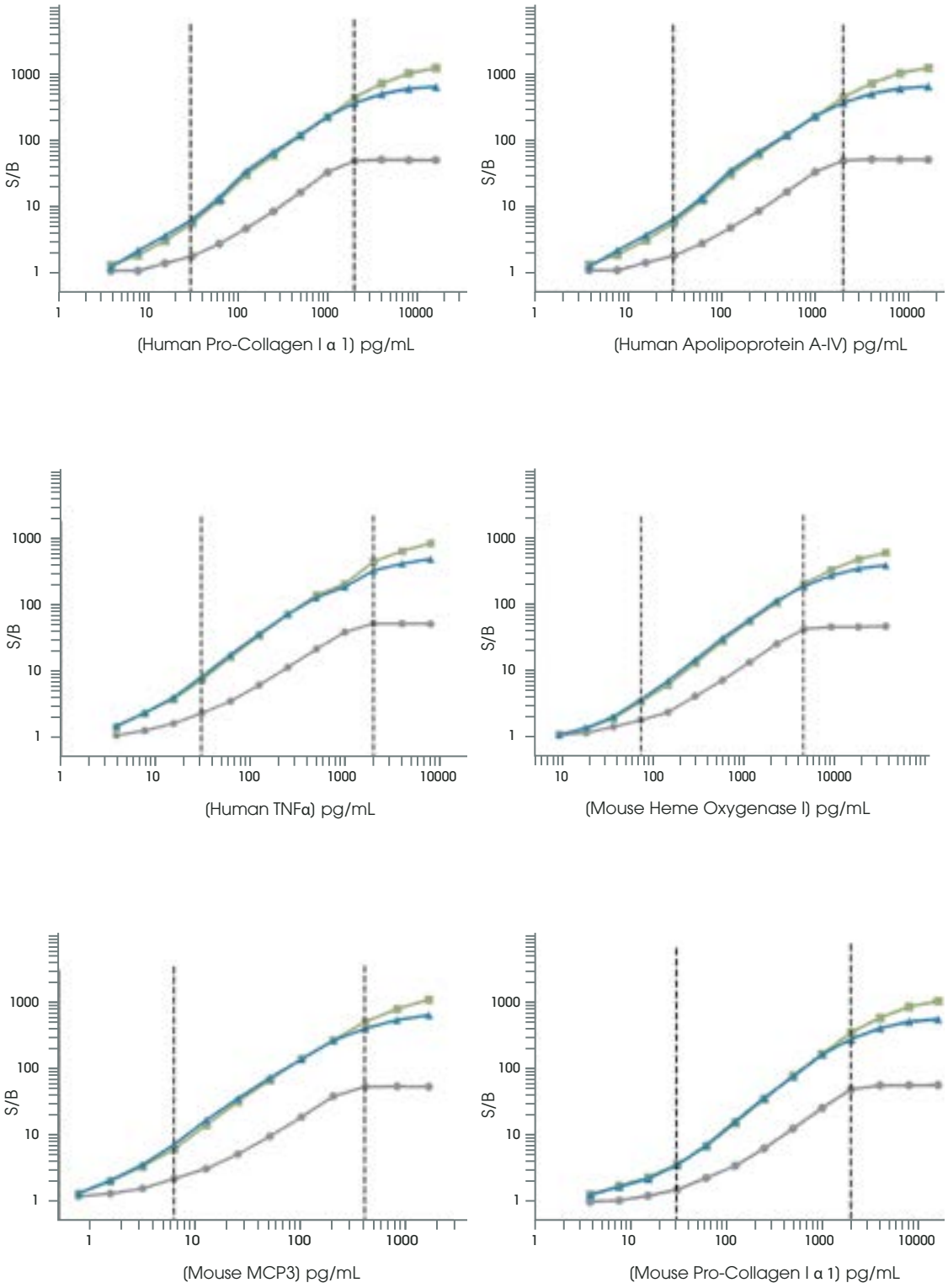

Figure 2. Comparisons of standard curves for SimpleStep ELISA absorbance kits vs. CatchPoint SimpleStep ELISA fluorescence kits read on a SpectraMax M4 reader. The colorimetric signal development was stopped after 10 minutes (gray plots), whereas the fluorescent signals were measured 10 minutes (green plots) or one hour (blue plots) after the Stoplight Red substrate was added. The graphs were plotted with signal-to-background ratio (S/B) vs. concentration (pg/mL). The dashed vertical lines denote the concentration ranges recommended for current absorbance kits. The dynamic range, both lower and upper, is extended with CatchPoint SimpleStep ELISA (fluorescent) kits.

Results

The data presented in Figure 2 demonstrate that fluorescent CatchPoint SimpleStep ELISA kits offer higher signal window and extended dynamic range compared to the SimpleStep ELISA absorbance assay kits. Results varied depending on the specific assay kit used. The CatchPoint SimpleStep ELISA kit signal-to-background ratio (S/B) was shown to be stable for at least one hour. Without a step to stop the signal development reaction, the fluorescent kits enable users to optimize time vs. signal for their own assays, as well as provide readout flexibility.

When the CatchPoint SimpleStep ELISA, or any fluorescence assay, results are detected on different fluorescence microplate readers, the magnitude of the signals obtained often varies. Plotting the results as signal/background, shown in Figure 3, yields nearly identical plots for all readers tested, confirming that any of the readers are suitable for detection of CatchPoint SimpleStep ELISA fluorescent signals.

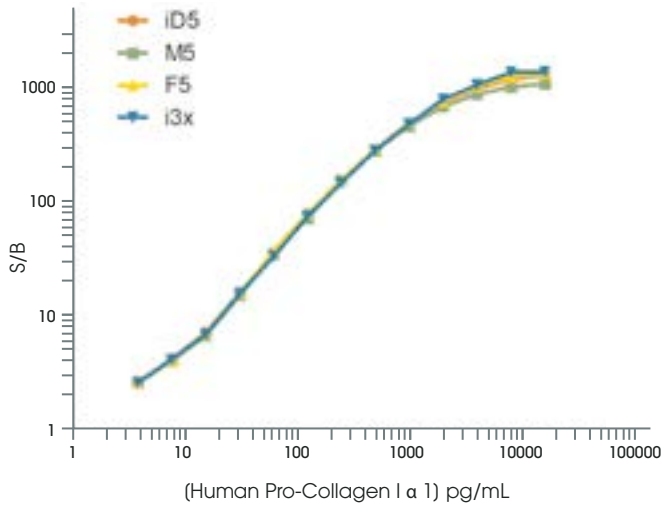

Figure 3. Data Comparison of fluorescent Human Pro-Collagen I alpha 1 CatchPoint SimpleStep ELISA kit using different plate readers. The same assay plate was read on SpectraMax iD5, SpectraMax i3x, SpectraMax M5, and FilterMax F5 readers using preconfigured protocols in SMP. Graphing normalized S/B vs. analyte concentration yields nearly identical plots for all four readers.

Conclusion

CatchPoint SimpleStep ELISA kits are fluorescence-based and offer increased signal and dynamic range over absorbance-based SimpleStep ELISA kits. CatchPoint SimpleStep ELISA kits give users a 90-minute ELISA workflow, a nearly two-thirds reduction in the time required to achieve results compared to conventional ELISAs. CatchPoint SimpleStep ELISA kits have been validated on SpectraMax and FilterMax readers, using preconfigured SoftMax Pro Software protocols that make it easy to detect the assay and get results quickly.

See the full list of compatible microplate readers at:
www.moleculardevices.com/products/assay-kits/elisa/catchpoint-simplestep-elisa-kits

www.abcam.com/simplestep