CYGNUS TECHNOLOGIES
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11 June 2024

Change Notification Memo: *Pichia pastoris* 3G HCP ELISA Kit, Item # F1015, Now Available

Importance: HIGH

Dear Customer,

This letter is to provide all current customers of our *Pichia pastoris* HCP ELISA Kit, 2G, Item # F640 [available since 2012], with notification of a resupply of the critical components used in this assay. Based on the current supply and demand of this kit, we expect the depletion of the current reagents to occur in June 2025. This notification is to inform you of the minimal overlap between the F640 and F1015 *Pichia pastoris* 3G HCP ELISA kits and to start the conversation to perform any bridging, re-qualification, or re-validation studies that you may deem necessary to transition to the new reagents. Kits manufactured using the new antibodies are now available so that you can begin bridging studies with Item # F1015.

Cygnus Technologies produced, characterized, and qualified large pools of the new capture and detection antibodies that will replace the current antibody lots. These antibodies have been generated through immunization against a new lot of *Pichia pastoris* HCPs derived from *Pichia* GlycoSwitch® strains designed for making glycoproteins with mammalian-like sugars. The immunization was conducted in the same way as was performed in generating the F640 reagents and the antibodies have been affinity purified using the same procedures as the original reagents. The coverage of the new antibodies to the *Pichia pastoris* HCP antigen was determined to be in the range of 88-100% by Antibody Affinity Extraction combined with Mass Spectrometry.

The following table compares F640 and F1015 assay design and specifications and highlights changes:

	F640	**F1015**
Capture Antibody	Goat	Goat
Detection Antibody	Goat	Goat
Reporter Enzyme	Horseradish Peroxidase	Horseradish Peroxidase
Antigen	*Pichia pastoris*	*Pichia* GlycoSwitch®
Standards Diluent	I028	I028
Conjugate Diluent	I056+additives	I056
Affinity Purified	Yes	Yes, identical procedure
Antibody coverage analysis as determined by AAE with 2D-PAGE	N/A	N/A
Antibody coverage analysis as determined by AAE-MS	N/A	88-100%
LOD	0.1 ng/mL	0.2 ng/mL
LOQ	~0.5 ng/mL	~3 ng/mL
Precision	Intra-assay: 3.0% -4.2% Inter-assay: 1.6% -2.8%	Intra-assay: 6.0%-7.3% Inter-assay: 0.0%-4.3%

Accuracy	80%-120%	91%-106%
Simultaneous protocol	Yes	Yes
Detection Ab and Sample/ Standard/Control incubation time	3 hours	1 hour
Standards provided	0, 1, 4, 20, 75, and 250 ng/mL	0, 3, 6, 12, 25, 50, 100, and 200 ng/mL
Shelf Life	6 months	6 months at launch, will be extended to 12 months pending completed real time stability study

We suggest at least the following studies be performed to qualify the F1015 kit and corresponding capture antibody:

1. Establish the mean and acceptable range for your controls with the F1015 kit. These values may be different (higher or lower) relative to the current antibody. To avoid failing runs due to 'out of specification' controls, it may be necessary to set a new range. If you use other curve parameters such as ODs as an indirect specification, these too may require a new range to be established.

2. Test in-process and drug substance samples using the F640 and F1015 kits in parallel to determine if there is a consistent and significant difference and bias (higher or lower values) from the F640 kit.

3. Perform dilution linearity and spike recovery on your samples with the new antibodies to assure accuracy and specificity.

4. Orthogonal determination of coverage is best determined using our AAE method. We recommend performing AAE on at least two samples: 1) an upstream harvest sample to determine coverage to the majority of the proteome and 2) a down-stream sample to determine coverage of those HCPs that persist through the purification process. Cygnus can perform the AAE analysis for you.

5. If you are using the F640 kit for lot release testing, determine what if any effect differences in control and sample values will have on your release criteria and document those changes. Changing the immunoreagents changes the originally validated specificity of the HCP ELISA for each product, regardless of whether the measured ppm levels are the same or not. That requires re-validation of immunoreactivity and updating of the HCP ELISA SOP before the new F1015 kits can be used for lot release testing of DS lots previously approved for testing with F640.

Kits using the new antibody are now available so that you can begin bridging studies.

Please forward a copy of this notification to those in your organization affected by this change.

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Cygnus Technologies strives to provide you with the highest quality products and services, and we hope that this notification will result in as little disruption to your processes as possible. If you have any questions or concerns, please email technical services at techsupport@cygnustechnologies.com and we will be pleased to arrange a teleconference to discuss the use of orthogonal testing to better understand any observed differences.

Sincerely,

Eric Bishop
VP of R&D