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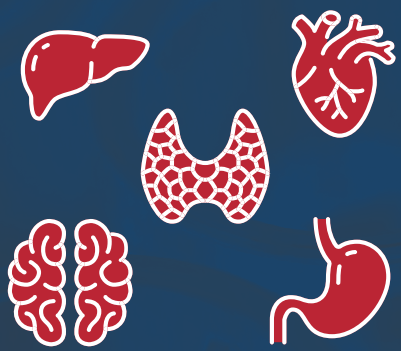
OVERCOMING DIAGNOSTIC ASSAY CHALLENGES

WITH RECOMBINANT PROTEINS

Although recombinant proteins have been available for biomedical applications since the early 1980s, the clinical diagnostic industry has been slow to make the move away from proteins purified from naturally occurring sources, including organs, tissues, and blood.[1,2]

Historically, native proteins performed better than recombinants and were in ample supply. However, scientists face present day hurdles, including pandemic- and war-driven supply disruptions, which jeopardize the diagnostic industry's ability to deliver life-saving assays. Recombinant proteins will be essential for the diagnostic industry to keep moving forward.[2]

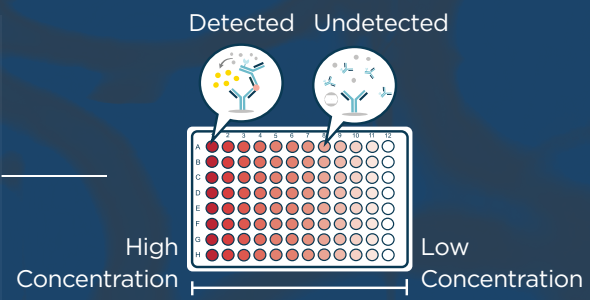


Availability

Proteins from biological specimens rely on starting material availability. Critical human- and animal-derived tissues are increasingly expensive and difficult to acquire, and quality has declined over the years. In contrast, researchers can depend on the availability and assurance of recombinant proteins, which are characterized by renewable starting material, short production lead-times, and batch-size scalability.[2]

Performance

The long-held opinion that recombinants do not function as well as native proteins is no longer true. Recent progress in recombinant technologies and purification strategies paved the way to recombinant proteins that meet and exceed the previous performance challenges, caused by structural complexity and post-translational modifications.[2,3]

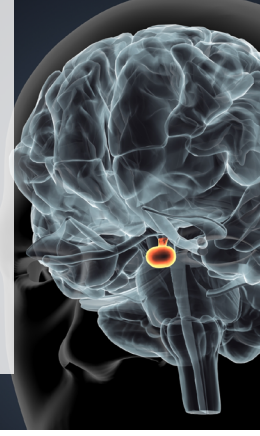


Reliability

A new era of reliable recombinant proteins has dawned, thanks to validation assays that establish protein integrity with multiple clinical analyzers and other antibody-based methods. Researchers can rely on recombinant proteins for a variety of systems, replacing native biomarkers in reproductive endocrinology, anemia and metabolism, cardiology, and beyond.[2]

Researchers can now turn to recombinant protein for the availability, performance, and reliability needed to overcome modern hurdles along the path to delivering diagnostic assays. From the starting blocks to the finish line, Scripps Laboratories is leading the way in developing novel recombinants that meet the needs of the diagnostic industry.[2]

RECOMBINANT HORMONES FOR DIAGNOSTICS: BEYOND TISSUE-DERIVED TOOLS



Recombinant forms of human hormones have been available for decades, yet, in the field of endocrinology, there is an evident lag in embracing recombinant proteins for diagnostic assays. Historically, recombinant forms of glycosylated, two-subunit pituitary hormones have not performed well enough to replace their native counterparts. New approaches to purification and improved emphasis on epitope assessment ensure that recombinant proteins perform in a range of antibody-based systems suitable for endocrine diagnostics.[1]

History of Pituitary Hormones in Diagnostics

Clinicians often turn to recombinant pituitary hormones as treatment options, including follicle stimulating hormone (hFSH), luteinizing hormone (hLH), and thyroid stimulating hormone (hTSH), because these recombinants have similar biological function as native hormones. However, immunoassays for monitoring and detecting pregnancy, fertility, ovulation, and thyroid conditions depend on more than biological function. For example, serum levels of hTSH are routinely measured with immunoassays as an indicator of thyroid function. These assays help doctors diagnose hyperthyroid syndromes (e.g., thyroid adenoma, nodular goiter, and the autoimmune disorder Graves' disease) and hypothyroidism (e.g., the autoimmune disease Hashimoto's thyroiditis). Such diagnostic immunoassays require epitope availability, which enables antibody-binding and hormone detection.[1-3]

Overcoming Performance Challenges

The diagnostic industry currently relies on hormones isolated from human donor tissues, glands, organs, and fluids because hormones are difficult to produce as recombinants. Scientists can purify recombinant hormones from cell lines grown and harvested on-demand. However, producing quality cell lines for recombinant production is merely the first step in this process, and it only partially addresses factors related to epitope presentation such as post-translational modifications (i.e., glycosylation) and heterodimerization.[1,2,4]

For instance, although recombinant hFSH and hLH have long been available from mammalian cell culture systems with mechanisms required for glycosylation, the historically poor performance of these recombinants in immunoassays has prevented their acceptance in the diagnostic industry. Because antibody-binding is key when assessing the suitability of a recombinant protein for immunoassay development, scientists must also employ purification techniques that assure proper protein folding, preserve glycosylation, and maintain epitope integrity.[1,2,4,5]

Thanks to new techniques that overcome these challenges, present-day recombinant hormones may be the solution to availability roadblocks in immunoassay diagnostics, as they are not limited by raw material supply chains. Based on the source and amount of protein needed, researchers may require hundreds, or even thousands, of individual donors for native proteins, which is not well suited for industrial-scale diagnostics. Native protein starting materials are subject to supply chain vulnerability and unpredictability, risk of infectious disease transmission, long production cycles that remove contaminating protein, and variable production yields.[2]

For immunoassay development, scientist must also employ purification techniques that assure proper protein folding, preserve glycosylation, and maintain epitope integrity.

Diagnostic Quality

With nearly forty years of protein purification expertise, Scripps Laboratories has implemented purification processes that maintain the structural integrity and antibody recognition of recombinant hormones. After purification, recombinant pituitary hormones are tested with several antibody-based assay systems, including ELISA, western blot, and multiple clinical analyzers using a variety of antibody pairs. This thorough assessment of antibody-based methods provides researchers with ample insight into epitope availability for a variety of systems and applications. In addition, expressing hormones without affinity tags provides proteins that are as close as possible to the native form.[1,2] Scientists can expect quality diagnostic assay performance from intact, unmodified recombinant pituitary hormones.

See references on page 6.

ESTABLISHING IMMUNOASSAYS WITH RECOMBINANT METABOLIC MARKERS

Recombinant proteins with complex, multi-subunit structures are among the most challenging to produce. Some proteins involved in metabolic function are known for their intricate structures, which prevent researchers from using recombinant metabolic proteins in diagnostic applications due to performance and supply limitations. Today, a novel line of recombinant proteins developed for metabolic diagnostics meets and exceeds the performance challenges of the past, ushering in a new frontier.[1]

Recombinant apoferritin and recombinant intrinsic factor are ready for use in research and clinical diagnostic assay development.

History of Metabolic Biomarkers in Diagnostics

Doctors routinely check serum levels of protein biomarkers as indicators of human health. Serum ferritin is a protein involved in iron homeostasis, and variations in serum levels detected by diagnostic immunoassays may indicate diseases such as iron-deficiency (anemia), inflammation, liver disease, and cancer. Intrinsic factor is a glycoprotein produced in the stomach that plays a crucial role in transporting and absorbing vitamin B12. Intrinsic factor levels can also be assessed by blood tests and competitive binding assays, with insufficiency causing a range of consequences, including blood, cardiovascular, and neurological disorders. Scientists rely on standardized protein controls for accurate diagnostics involving these biomarkers. In recent years, the limitations of human- and animal-sourced proteins have significantly affected such tests.[2-4]

Overcoming Supply Bottlenecks

Conventionally, researchers derive human ferritin from liver or spleen tissue after it is processed in bulk volumes to obtain sufficient yields for the clinical diagnostic industry. This process is arduous and dependent on donor organs, which are not always available. The unreliable availability of these tissues threatens to disrupt the long-term supply of purified ferritin. Similarly, vitamin B12 clinical diagnostic assays have traditionally relied on intrinsic factor sourced from pigs, which has been in short supply in recent years. A lengthy and outdated extraction process that was used for decades underwent a change several years ago, due to environmental and health concerns surrounding the process. The new process adversely affects the quality and yield of intrinsic factor, disrupting the steady supply for the clinical diagnostic industry.[2]

With native raw materials in increasingly short supply, scientists in the diagnostic industry must adapt. The transition to recombinant proteins may seem daunting, complicated, and time-consuming. Undoubtably, the validation processes, both internal and external, must thoroughly demonstrate that a recombinant product can replace a human- or animal-sourced protein. To this end, Scripps Laboratories has produced and vetted ground-breaking recombinant metabolic proteins, developed specifically for use in the clinical diagnostic industry.[1,2]

Meeting and Exceeding Diagnostic Standards

Recombinant apoferritin and recombinant intrinsic factor are ready for use in research and clinical diagnostic assay development. The recombinant form of the 24-subunit human apoferritin (ferritin without iron) is comprised of both the heavy and light chain ferritin subunits. When compared with native ferritin by SDS-PAGE, western blot, HPLC, and clinical immunoanalyzer assays, the similarities in physical characteristics and assay reactivity indicate that recombinant human apoferritin is a suitable and sustainable replacement for ferritin sourced from human liver and spleen. The recombinant form of human intrinsic factor is produced in a mammalian cell line and is glycosylated. Furthermore, it is highly purified and has demonstrated excellent B12-binding capability. Comparison of recombinant and porcine-sourced intrinsic factor established that this new recombinant form is a suitable replacement for animal-derived starting material.[2,5,6]

Native raw materials for metabolic diagnostic assays have been in short supply for several years and supply chain disruptions in the diagnostic industry are expected to continue for human glands, tissues, and organs. Recombinant metabolic proteins for clinical diagnostic applications are excellent solutions for the steady, long-term supply of biomarkers that exceed expectations and challenge what clinical diagnostic manufacturers can expect from recombinant proteins.

See references on page 6.

SCALE-UP CARDIAC DIAGNOSTICS WITH RECOMBINANT PROTEIN MARKERS

Cardiovascular disease is a major healthcare burden worldwide, and early detection is crucial yet challenging. Scientists turn to existing biomarkers as critical diagnostic tools in cardiovascular health and look toward new biomarkers that improve cardiovascular disease detection.[1] However, cardiovascular biomarker research is hindered by the current scale of protein purification from human tissues or serum. Recombinant biomarkers offer a key future avenue in the cardiovascular diagnostic industry, not only as suitable replacements for native proteins, but also as more reproducible biomarkers that can be rapidly produced.

History of Cardiovascular Biomarkers in Diagnostics

When developing biomarker tests, scientists use cardiac-restricted epitopes within proteins such as troponin T and creatine kinase MB (CK-MB) for antibody-based diagnostics. Creatine kinase is a dimeric enzyme composed of B or M subunits that are encoded by their own unique genes. The M and B subunits combine to form three different isoenzymes (CK-BB, CK-MB, and CK-MM), which are expressed in various human tissues. For instance, CK-BB is predominantly in brain tissue, while CK-MB is in heart muscle, and CK-MM is in both skeletal and heart muscle. Similarly, different forms of troponin proteins are found in skeletal and cardiac tissue. Because these forms are immunologically distinct proteins, researchers develop monoclonal antibody-based tests that distinguish between heart-specific biomarkers and those from other tissues.[1-3]

For instance, heart attack biomarker panels often include immunoassays that measure CK-MB concentration in the blood, as well as myoglobin and troponin I and T.[3,4] Usually, troponin I and T are not detectable in the blood of healthy people, and an increase in cardiac-specific troponin proteins is regarded as a gold-standard marker for heart attacks.[5] Elevated CK-MB can be detected within three to four hours of heart attack onset, however high-sensitivity troponin detection assays provide more value in early and late diagnosis. Still, assays that examine CK-MB plasma levels remain useful for diagnosing additional infarctions in individuals who previously experienced a heart attack.[1,3]

Overcoming Starting Material Scarcity

Researchers have developed and continue to hone high-affinity assays that detect cardiac biomarkers. Currently, scientists seeking to establish accurate and specific diagnostic immunoassays depend heavily on raw materials such as human hearts and serum. Large-scale purification from such precious and limited starting materials is a problem area in research tool development due to cost and underabundance. This dilemma predates COVID-19, but the pandemic exacerbated the scarcity and high cost of these materials. In contrast, renewable, responsibly sourced, recombinant protein provides a steady, long-term supply of cardiac biomarkers for clinical diagnostics.[2,6]

Renewable, responsibly sourced, recombinant protein provides a steady, long-term supply of cardiac biomarkers for clinical diagnostics.

Making the Smart Choice for Cardiovascular Diagnostic Development

Recombinants are sourced from cell lines that are grown and harvested on-demand so researchers can access the purified protein when needed and in the large quantities required by the diagnostic industry. Because cardiovascular disease biomarker panels rely on heart-restricted epitopes for specificity, recombinant biomarkers must use the proper gene sequence, avoid affinity tags, and maintain protein structure and integrity during purification. These considerations will ensure that recombinant proteins are not only comparable to native proteins in clinical diagnostic assay development, but are virtually indistinguishable from or superior to proteins sourced from human tissues and fluids.[6,7]

Recombinant troponin T is a reliable and economical alternative to its native counterpart. Its suitability for research use, immunoassay development, and large-scale diagnostic assay manufacturing has been experimentally established. Additionally, recombinant troponin T expressed in *E. coli* enables large-scale production, unlike native troponin T, which typically has low yields.[8]

Recombinant proteins are the smart choice now and for future diagnostic approaches. For researchers focused on cardiovascular diagnostics and assay development, Scripps Laboratories has recombinant troponin T available now and will have recombinant CK-MB and troponin I available soon.

See references on page 6.

References

Infographic: Overcoming Diagnostic Assay Challenges with Recombinant Proteins

1) P.V. Pham, "Medical biotechnology: techniques and applications," *Omics Technologies and Bio-Engineering: Towards Improving Quality of Life*, D. Barh, V. Azevedo, eds., London: Academic Press, 2018, p. 449-469.
2) "Why recombinants are the future of diagnostics," *Scripps Laboratories*, https://scrippslabs.com/content/recomb-future-of-dx.pdf, accessed March 29, 2023.
3) N.K. Tripathi, A. Shrivastava, "Recent developments in bioprocessing of recombinant proteins: expression hosts and process development," *Front Bioeng Biotechnol*, 7:420, 2019.

Article 1: Recombinant Hormones for Diagnostics: Beyond Tissue-Derived Tools

1) "Recombinant hormones," *Scripps Laboratories*, https://scrippslabs.com/recombinant-hormones/, accessed March 29, 2023.
2) "Why recombinants are the future of diagnostics," *Scripps Laboratories*, https://scrippslabs.com/content/recomb-future-of-dx.pdf, accessed March 29, 2023.
3) "TSH background," *Scripps Laboratories*, https://scrippslabs.com/tsh-background/, accessed March 29, 2023.
4) A. Olivares et al., "Reactivity of different LH and FSH standards and preparations in the World Health Organization matched reagents for enzyme-linked immunoassays of gonadotrophins," *Hum Reprod*, 15(11):2285-91, 2000.
5) M.T. C.P. Ribela et al., "Synthesis and chromatographic purification of recombinant human pituitary hormones," *J Chromatogr B Analyt Technol Biomed Life Sci*, 790(1-2):285-316, 2003.

Article 2: Establishing Immunoassays with Recombinant Metabolic Markers

1) "Why recombinants are the future of diagnostics," *Scripps Laboratories*, https://scrippslabs.com/content/recomb-future-of-dx.pdf, accessed March 29, 2023.
2) "Recombinant metabolic markers," *Scripps Laboratories*, https://scrippslabs.com/recombinant-metabolic-markers/, accessed March 29, 2023.
3) Y. Hu et al., "Identification of serum ferritin-specific nanobodies and development towards a diagnostic immunoassay," *Biomolecules*, 12(8):1080, 2022.
4) H.M. Al-Awami et al., "Physiology, gastric intrinsic factor," *StatPearls [Internet]*, Treasure Island, Florida: StatPearls Publishing, 2023, https://www.ncbi.nlm.nih.gov/books/NBK546655/, accessed March 29, 2023.
5) "Technical brief: Recombinant apoferritin vs. native ferritin," *Scripps Laboratories*, https://scrippslabs.com/content/TB-Rec-Apoferritin-r1.pdf, accessed March 29, 2023.
6) "Technical brief: Recombinant human intrinsic factor," *Scripps Laboratories*, https://scrippslabs.com/content/TB-Rec-IntrinsicFactor-r1.pdf, accessed March 29, 2023.

Article 3: Scale-up Cardiac Diagnostics with Recombinant Protein Markers

1) F. Omran et al., "Cardiovascular biomarkers: lessons of the past and prospects for the future," *Int J Mol Sci*, 23(10):5680, 2022.
2) K.C. Park et al., "Cardiac troponins: from myocardial infarction to chronic disease," *Cardiovasc Res*, 113(14):1708-18,2017.
3) "Creatine kinase background," *Scripps Laboratories*, https://scrippslabs.com/creatine-kinase-background/, accessed March 30, 2023.
4) E. Danese, M. Montagnana, "An historical approach to the diagnostic biomarkers of acute coronary syndrome," *Ann Transl Med*, 4(10):194, 2016.
5) R. Xu et al., "High-sensitive cardiac troponin T," *J Geriatr Cardiol*, 10(1):102-9, 2013.
6) "Recombinants for research & diagnostic applications," *Scripps Laboratories*, https://scrippslabs.com/recombinants-for-research-diagnostic-applications/, accessed March 30, 2023.
7) "Recombinant cardiac markers," *Scripps Laboratories*, https://scrippslabs.com/recombinant-cardiac-markers/, accessed March 30, 2023.
8) "Technical brief: Recombinant troponin T," *Scripps Laboratories*, https://scrippslabs.com/content/TB-Rec-TnT-r1.pdf, accessed April 19, 2023.