SpearBio

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SPEAR UltraDetect™

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Successive Proximity Extension Amplification Reaction (SPEAR) Technology

Ultra-sensitive immunoassays are critical tools for detecting low-abundance protein biomarkers in biological matrices in research and clinical applications. Technologies like electrochemiluminescence immunoassay (ECLIA), digital ELISA, and nucleic acid linked immuno-sandwich assay (NULISA™) have shown to reach femtomolar or even attomolar-level sensitivity. However, these heterogeneous assay technologies require the use of solid surface capture and suffer from limitations such as reliance on high-affinity antibodies, non-specific binding to solid surface, high variations due to complex workflows, and proprietary instrumentation.

SPEAR is a novel immunoassay technology that overcomes the sensitivity limitations of conventional heterogeneous immunoassay technologies. By integrating nucleic acid amplification into a unique two-factor authentication mechanism for protein detection, SPEAR enables highly specific and **robust** protein detection in a fully **homogeneous**, wash-free format.

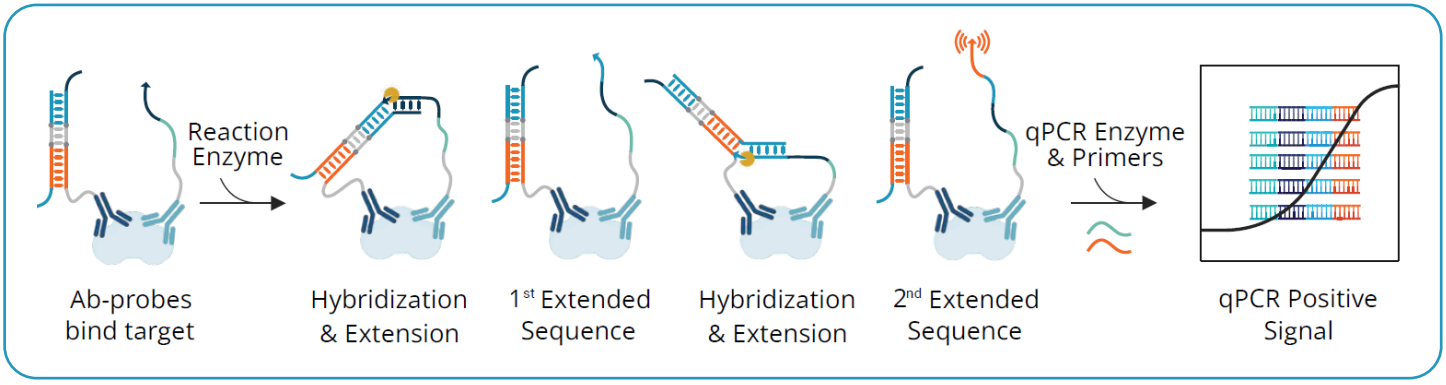
This innovative approach delivers the first ultra-sensitive immunoassay platform that combines enhanced specificity, workflow simplicity, and minimal sample input, while leveraging standard qPCR for readout.

Mechanism and benefits of SPEAR technology

SPEAR uses two target-specific antibodies, each linked to a unique DNA device. One is mainly double-stranded with two toeholds; the other is single-stranded and carries a short sequence complementary to one toehold. Upon target binding, proximity enables hybridization and polymerase extension, adding a first extended sequence on the single-stranded DNA device.

A second hybridization-extension step occurs only when the probes remain co-localized for an extended period, creating an amplifiable DNA sequence. This **two-factor authentication** minimizes background and non-specific signal, delivering unparalleled **sensitivity** and **specificity**. Target-specific signals are quantified through standard qPCR, with robust consistency across a wide range of qPCR systems.

SPEAR's Two-Factor Authentication Mechanism



A unique homogeneous immunoassay technology

The homogeneous nature of SPEAR eliminates non-specific binding associated with solid surface capture, further enhancing specificity. Because the reaction occurs entirely in solution, SPEAR requires no wash steps, thus simplifying the workflow, enabling easy automation and further augmenting **precision**, demonstrating mean CVs <5% from as little as **1 µL of diluted sample**.

SPEAR UltraDetect™ Assays

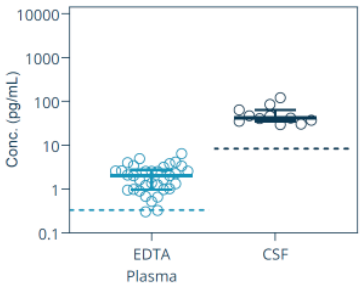
Disease-Specific Applications

SPEAR UltraDetect pTau 231

- Superior sensitivity with over 97% quantifiability, facilitating earlier detection of disease
- High precision, enabling tracking of disease progression in longitudinal studies

Assay Specification *

fLLoD	0.157 pg/mL
fLLoQ	0.330 pg/mL
Mean CV intra-assay (inter-assay)	2.8% (6.5%)
Healthy Control Above LLoD	100% (n=34)
Healthy Control Above LLoQ	97% (n=34)



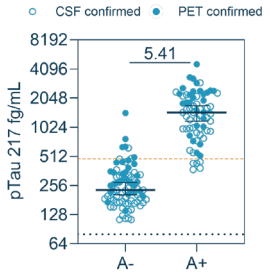
Endogenous reading of pTau231: 97% and 100% above fLLoQ in plasma (n=34) and CSF (n=11) samples, respectively.

SPEAR UltraDetect pTau 217

- Greater specificity resulting in larger fold difference in distinguishing amyloid positives and negatives
- High consistency and precision, empowering large-scale and longitudinal studies

Assay Specification*

fLLoD	19.7 fg/mL
fLLoQ	80.0 fg/mL
Mean CV intra-assay (inter-assay)	3.3% (4.4%)
Healthy Control Above LLoD	100% (n=35)
Healthy Control Above LLoQ	100% (n=35)



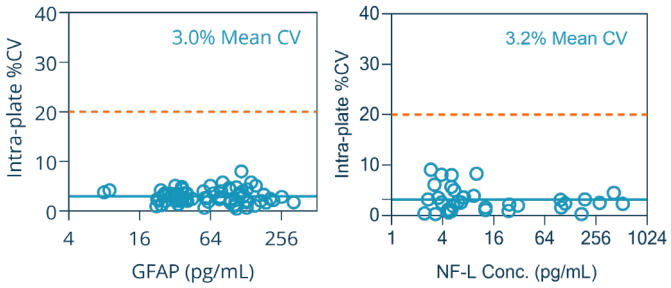
A 5.41x difference between mean pTau217 concentrations of AD (n=40) and non-AD (n=48).

Broader Neurology Applications

SPEAR UltraDetect GFAP
SPEAR UltraDetect NF-L

- Highly sensitive and precise measurement of GFAP and NF-L for a wide range of neurological conditions
- Broad dynamic range for GFAP ensuring accurate quantification from baseline to high-level concentrations.

Assay Specification*	GFAP	NF-L
fLLoD	0.163 pg/mL	0.096 pg/mL
fLLoQ	0.625 pg/mL	0.500 pg/mL
Dynamic Range	0.625-20,000 pg/mL	0.5-4,000 pg/mL
Mean CV intra-assay (inter-assay)	1.4% (4.8%)	3.2% (5.4%)
Healthy Control Above LLoD	100% (100%) n=21 (n=15)	100% (100%) n=10
Healthy Control Above LLoQ	100% (100%) n=21 (n=15)	100% (100%) n=10



Intra-plate CVs of GFAP (n=55) and Nf-L (n=42).

*Data are generated from using 1 µL of plasma or serum samples diluted at 4x. Mean CV data are based on measurements from samples representing low, medium, and high concentrations ran in duplicates across a minimum of 6 runs.

SPEAR UltraDetect™ Assay and Reaction Kit Configuration

Assay Kits Contain:
- **Assay-specific probes**
- **Calibrator**
- **Diluents**

Sufficient for one 96-well plate or half of one 384-well plate

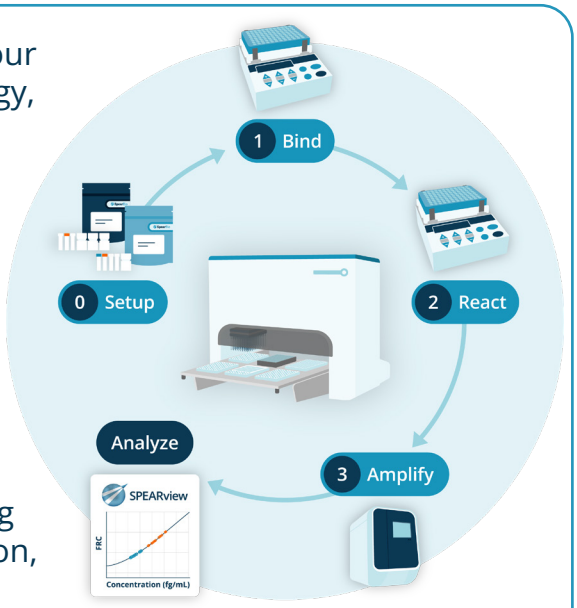
Reaction Kit Contains:
- **Universal reaction enzymes & buffer**
- **Universal qPCR reagents**
- **Sealing oil**

*Sufficient for **four** 96-well plates or **one** 384-well plate*

Assay and Reaction Kits combine to provide all workflow-required reagents

The SPEAR Solution for Protein Biomarker Detection

The SPEAR solution integrates reagent kits powered by our homogeneous, two-factor authenticated assay technology, with automated liquid handling, incubation, standard qPCR, and data analysis software.



1. Assay & Reagent Kits
Our growing menu of SPEAR UltraDetect™ Assay kits and companion Reaction Kit include all required reagents and easy-to-follow instructions for sample preparation and assay execution.

2. Semi-Automated Assay Workflow
The Formulatrix® F.A.S.T™ automates all critical pipetting steps, incuding a calibrator dilution series, sample dilution, and reagent additions.

The SPEAR Bioshake iQ comes with pre-programmed settings to ensure optimal temperature and mixing at key stages of the workflow.

3. Readout and Data Analysis
SPEAR's unique two-factor authentication mechanism generates a true binding signal for read-out on standard qPCR. The SPEAR UltraDetect Solution is compatible with the majority of in-stalled base of major qPCR systems.

Easy-to use **SPEARview** software transforms qPCR data into sample concentration results and provides seamless support across different qPCR formats.

Experiencing SPEAR UltraDetect™

The SPEAR UltraDetect Solution is available through our distribution partner, **Bio-Techne.**

Assess SPEAR UltraDetect with Your Samples

Spear Bio offers SPEAR Evaluation Services for researchers interested in assessing the SPEAR UltraDetect Solution before adoption. The service includes pre-testing consultation and data report and review led by a Spear Bio scientist.

Contact Us!

Request pricing or connect with a Bio-Techne specialist to learn more at:
www.bio-techne.com/spear-info

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