

August 13, 2018

Dr. Avital Braiman  
Director of Editorial  
*Journal of Visualized Experiments*  
1 Alewife Center, Suite 200  
Cambridge, MA 02140

Dear Dr. Braiman:

I am pleased to submit an original research protocol entitled "Detection of Proteases by Fluorescent Peptide Zymography", by Ameya Deshmukh, Jessica Weist and Jennifer Leight, for consideration for publication in the *Journal of Visualized Experiments*.

In this manuscript, we describe the protocol for a modified zymographic technique in which we covalently link fluorescent peptides to polyacrylamide gel matrices using an azido-PEG3-maleimide crosslinker. This manuscript provides an in-depth protocol as a companion to our recently published article in *Biotechniques*. This technique integrates degradable moieties through a covalent reaction into a zymogram gel. This covalent incorporation is a critical feature of this method, which now enables incorporation of a vast array of degradable substrates. Covalent attachment overcomes the susceptibility of these substrates to diffuse out of the gel during electrophoresis or development. The modified gels were used to measure the activity of various proteases in purified samples as well as in conditioned cell media, enabling detection of proteases not currently observed with gelatin zymography. Fluorescent peptide zymography greatly expands the library of proteases that can be detected and presents a technique for improving the sensitivity of current methods through design of new substrates.

We believe that this manuscript is appropriate for publication by the *Journal of Visualized Experiments* because it outlines an original technique which researchers in various disciplines can use to separate proteases by molecular weight and detect their activity. Fluorescent peptide zymography can complement current efforts aimed at elucidating the roles of proteases in disease, development and wound healing.

We have no conflicts of interest to disclose.

Thank you for your consideration!

Sincerely,



Jennifer L. Leight, Ph.D.  
Assistant Professor of Biomedical Engineering  
The Ohio State University