XENOTECH
A BioIVT Company

Human Lysosomes

XenoTech Offers Human Liver Lysosomes for Biopharmaceutical Development...

Lysosomes

Lysosomes are membrane-bound cellular organelles that are the site of degradation / catabolism, including extracellular substrates (endocytic pathway) and intracellular substrates (autophagy pathway). Human hepatic lysosomes contain high concentrations of lysosomal degradative enzymes, constituting an advantageous test system for analysis of catabolism and/or activation for targeted biopharmaceuticals that enter cells through the endosomal-lysosomal pathway.

Lysosomes are a convenient *in vitro* drug development tool to cost-effectively evaluate lysosomal stability of biopharmaceuticals and macromolecules. The data provided by these systems is used to direct development of biologics, such as ADCs, siRNA/RNAi molecules, immunotherapeutics, biodegradable copolymers and nanoparticles, etc.

Test System Advantages

Purified liver lysosomes show superior lysosomal enzymatic activity when compared to liver homogenate or S9 fraction, with low contaminating activity from mitochondrial enzymes. Human hepatic lysosomes are more predictive *in vitro* reagents than individual purified proteins, as multiple enzymes or physical characteristics synergistically affect stability of biomolecules. For instance, ADCs are likely to have a heterogeneous drug antibody ratio (DAR) and targeted modifications that stabilize the compound while in circulation. Differing amounts of drug, conjugated to various regions of the antibody, with assorted types of linkers will undoubtedly affect ADCs' stability. Therefore, it is beneficial to conduct initial screens with this simple and *in vivo*-relevant system.

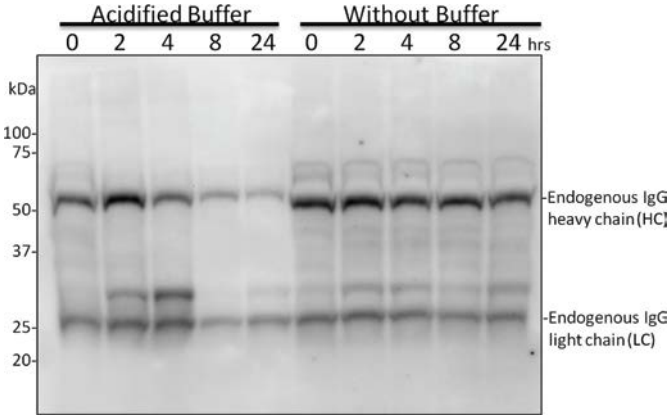
XenoTech's lysosomal isolation protocol has been optimized through characterization by western blotting, enzymatic activity and protease content.

XenoTech's offers human liver lysosomes and rat liver tritosomes as standard products and custom preparations from other species are made on-demand.

Catabolic Activity of XenoTech Lysosomes (continued on back)

Figure 1. Lysosomal catabolism of an endogenous IgG

Catabolism of endogenous human IgG that is ubiquitously present throughout the endosomal/lysosomal pathway in primary tissue was assessed in isolated lysosomes. Isolated lysosomes were acidified with XenoTech lysosomal catabolism buffer and incubated at 37°C. At indicated time points, samples were taken from the total reaction mixture and immediately heat inactivated. The samples were separated by SDS-PAGE, transferred to PVDF membranes, and immunoblotted with a HRP-conjugated goat anti-human IgG H&L (Abcam) to examine the endogenous IgG peptides. Bands were visualized using enhanced chemiluminescence with a FluorChem M imager (protein simple). Catabolism of the endogenous IgG was detected within 2 hours with the appearance of an IgG degradation product of 28-30 kDa. This band intensified at 4 hours and decreased to near undetectable level at later time points. The appearance and disappearance of this catabolite was accompanied by decreases in bands attributed to the IgG heavy and light chains. Reactions that were not acidified showed negligible IgG catabolism (right side of the blot). These data indicate that isolated hepatic lysosomes are an appropriate test system to analyze degradation of IgGs.



Contact us to learn more at **www.xenotech.com** or call **913.438.7450**

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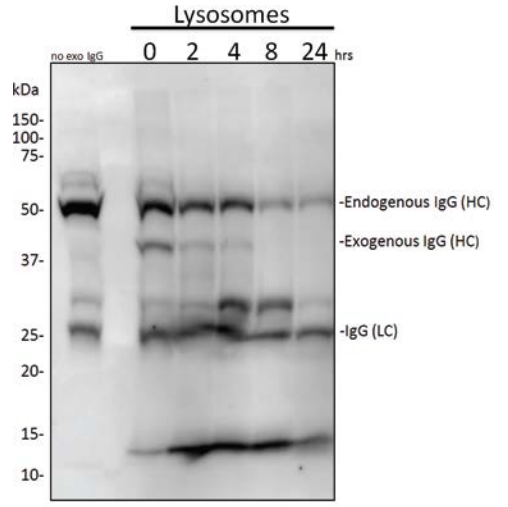

Figure 2. Lysosomal catabolism of an exogenous IgG

Lysosomal reactions were spiked with a purified human myeloma IgG1K (Ancell Corp) and carried out as described in Fig. 1. The heavy chain of this particular IgG runs at approximately 40 kDa with SDS-PAGE so its degradation can be observed without interference of heavy chain of the endogenous IgG (50 kDa). Catabolism of the IgGs was detected within 2 hours and coincided with an increase in intensity of the 12 kDa band. Over the course of the experiment, the exogenous and endogenous heavy chain bands decreased and were accompanied by decreases in higher and lower molecular weight bands throughout the sample. The detection of the expected 28-30 kDa IgG catabolite followed a time course observed in previous experiments (Fig. 1). The 12 kDa catabolite levels appeared to be fairly stable up to 8 hours, but by 24 hours decreased significantly. These data indicate that the catabolic capacity of the isolated lysosomes was not saturated by the endogenous cargo and that these isolations will catabolize additional biotherapeutics.

Figure 3. Titration of lysosomal catabolic activity with an exogenous IgG

Since the above presented data indicated that isolated lysosomes will catabolize endogenous and exogenous IgG, follow up experiments were carried out to titrate the catabolic capacity of the isolated lysosomes with exogenous IgG. For these experiments lysosomes were diluted to indicated protein concentrations and spiked with exogenous IgG. Catabolism experiments were incubated at 37°C for 4 hours and processed and analyzed as previously described. Lysosomes can be diluted to at least 2.25 µg per reaction (0.125mg/ml) without significantly diminishing the ability to degrade 135ng of exogenous IgG to near undetectable levels within 4 hours. Addition of a protease cocktail (Sigma Aldrich) to the catabolic reaction immediately after spiking with exogenous antibody blocked the catabolism of IgG, indicating that the disappearance of IgG was protease specific and can be inhibited by common protease inhibitors.

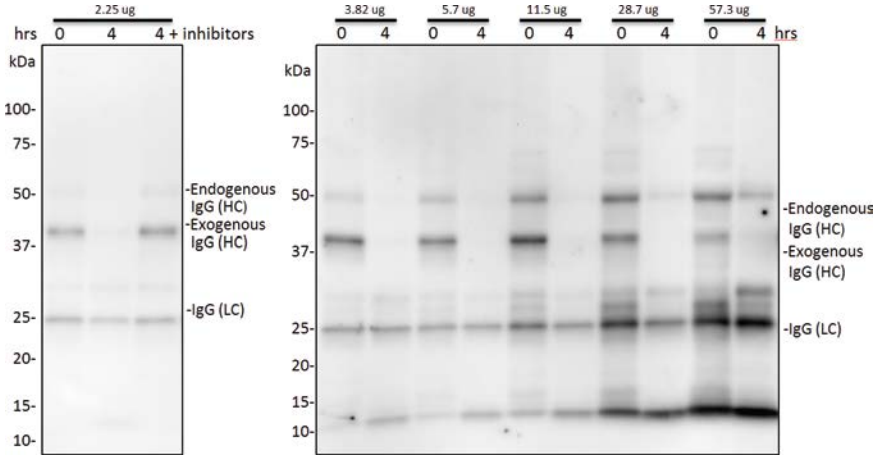

Figure 4. Comparison of catabolic activity of human hepatic S9 fraction vs. isolated lysosomes

To compare catabolic activity and efficiency of hepatic S9 proteins with isolated lysosomes, proteins were diluted to 1.0mg/ml and 0.125mg/ml respectively (Bessire, A et al. 2016). At these protein concentrations, Cathepsin B activity is very similar between fractions (data not shown). Catabolic reactions with exogenous IgG were carried out as previously described. The relative levels of IgG heavy chain detected in each sample were quantified by densitometry using ImageJ software. Lysosomes catabolized approximately 74% of the input IgG within 4 hours, while acidified S9 was only able to catabolize approximately 40% of the IgG (bar graph). These data indicate that despite very similar Cathepsin B specific activities, lysosomes are more efficient at catabolizing IgG and suggests that other enzymes contribute to degradation of the IgGs. Combined, these data suggest that acidified S9 may not offer a fully representative in vitro model of lysosomal catabolism. Representative immunoblots show the S9 and lysosomal IgG degradation. IgG catabolism data were from 3 independent catabolism experiments.