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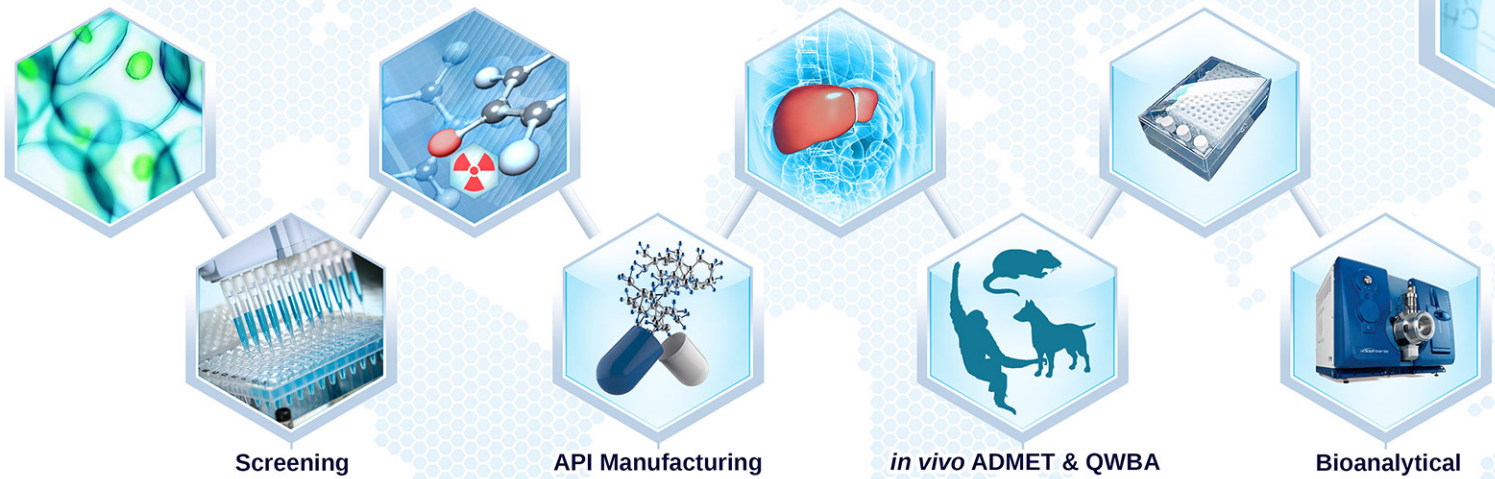
PROVEN GLOBAL CONTRACT RESEARCH EXPERTISE FROM DISCOVERY THROUGH CLINICAL SUPPORT



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Welcome to the webinar...

We will begin shortly

Investigation of freshly purified rat tritosomes and human hepatic lysosomes as an *in vitro* tool for characterization of biologic drugs

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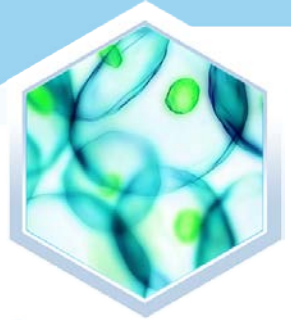


Lysosome Background

Discovered and named by Christian de Duve (Nobel Prize in 1974)

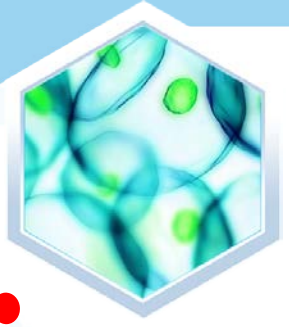
Lysosomes are a membrane bound, cellular organelle that is the sight of degradation/catabolism.

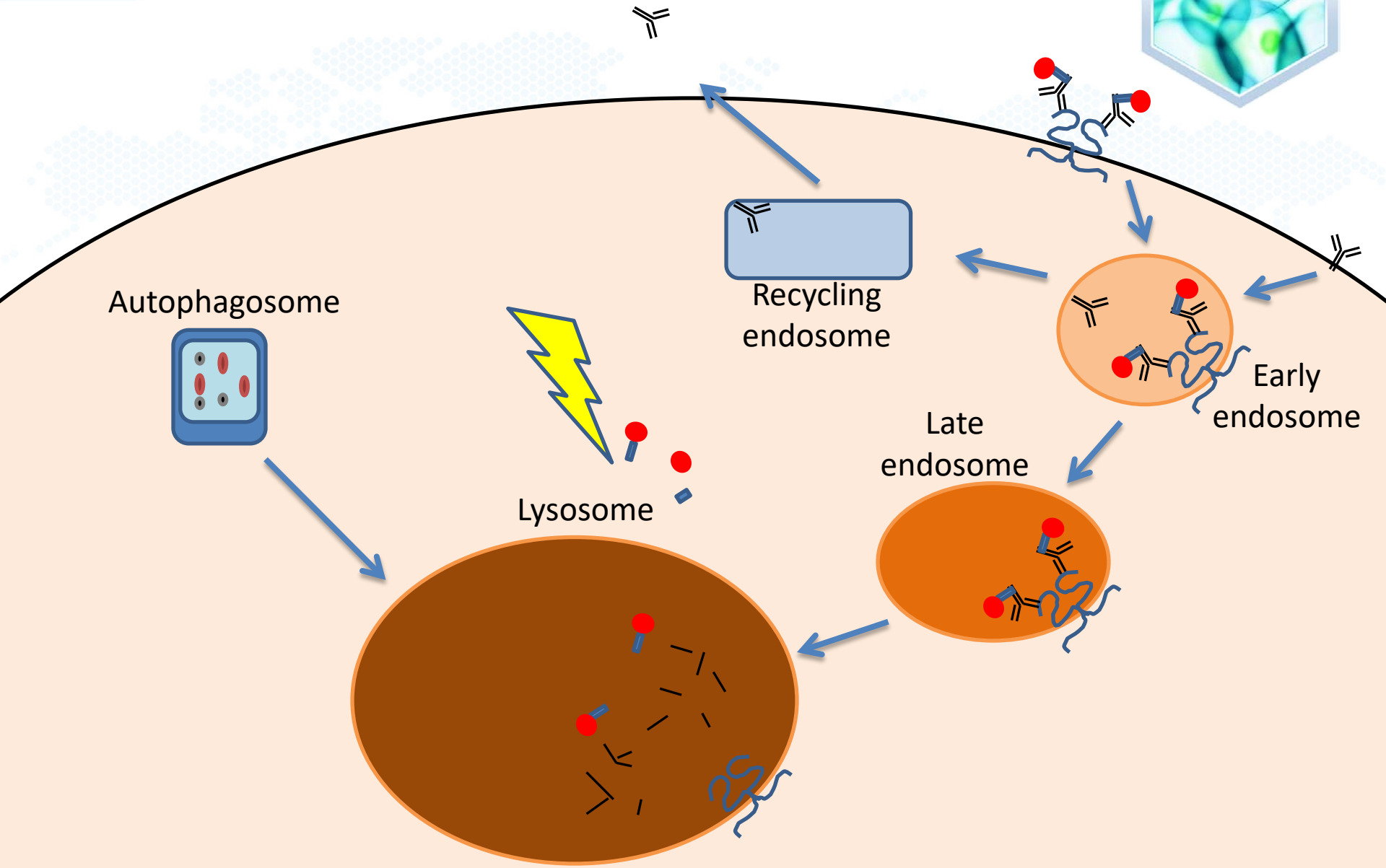
- extracellular substrates (endocytic pathway)
- intracellular substrates (autophagy pathway)

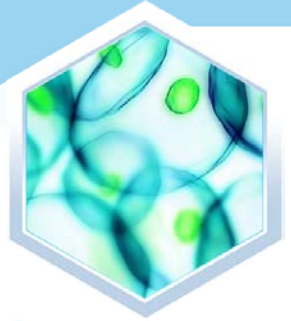
Contain a multitude of acidic hydrolytic enzymes and vary greatly in size.

Lysosomes contain a variety of catabolic enzymes and are being designed as the first site of catabolism/activation of targeted biopharmaceuticals that enters cells through the endosomal-lysosomal pathway.

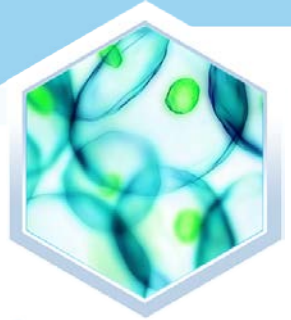
Isopycnic densities similar to mitochondria.




Autophagosome
Recycling endosome
Early endosome
Late endosome
Lysosome



Rat Tritosomes



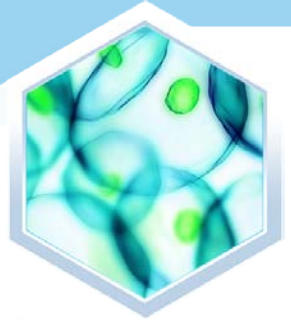
What are Tritosomes?

Tritosomes comes from the use of **Trito**n WR 1339 (now called Tyloxapol) to modify Lyso**somes** density.

Method was first described in the 60's and was developed due to overlapping densities between lysosomes and mitochondria in sucrose (most common density gradient material at the time of tritosome development).

Tyloxapol is trafficked to the lysosomes and results in altered lysosomal lipid composition. Combined with the uptake and sequestration of the Tyloxapol, the Tritosomes have a lighter density than untreated lysosomes and allows improved separation from mitochondria using sucrose density gradients.

Common technique used in research to purify and enrich lysosomes.



What are Tritosomes?

Highly purified lysosomes

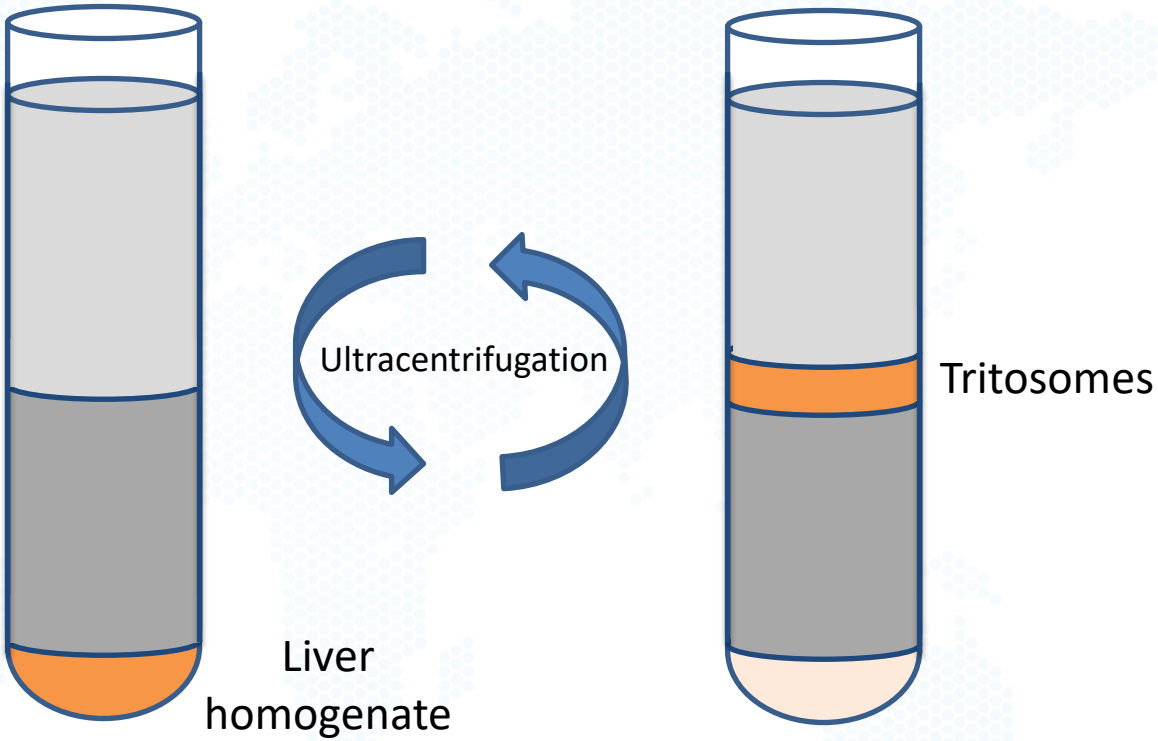
High specific activity of lysosomal enzymes and activities

- -Acid phosphatase
- -Cathepsin B
- -RNase

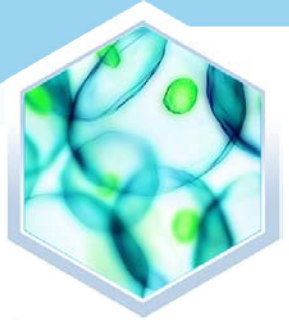
Simplified *in vitro* system

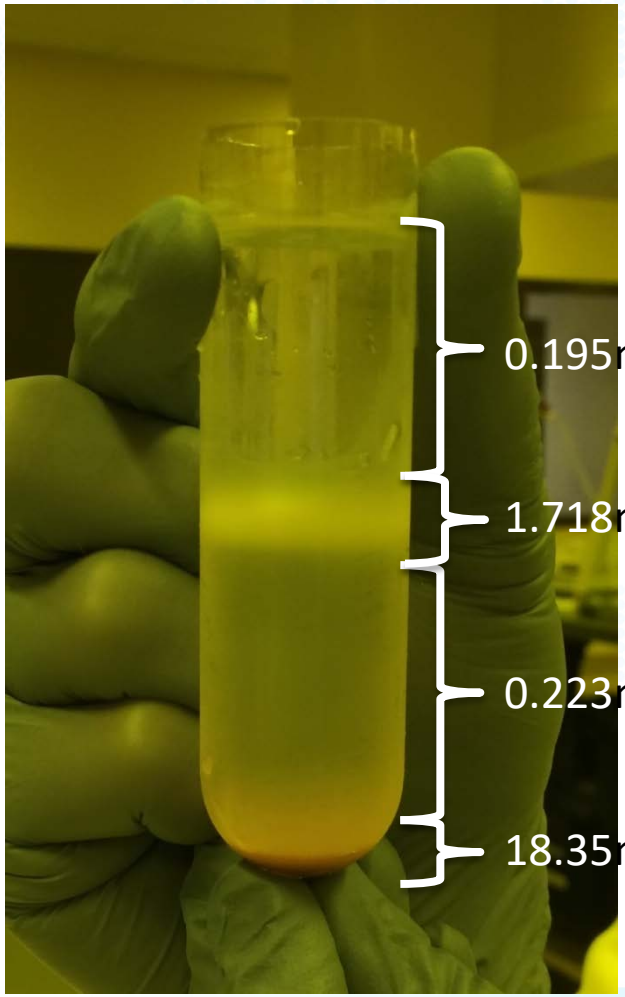
Tritosomes are disrupted and are ready- to – use
(EDTA and protease inhibitor free)

Tritosomes Production and Isolation



Tritosome Purity





Initial Liver Homogenate
34.05mg/ml Cat B = 1.43 U/mg COX = 0.0367 U/mg

0.195mg/ml Cat B = below detection level COX = 5.104U/mg

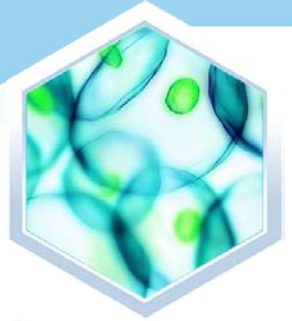
1.718mg/ml Cat B = 212.4 U/mg COX = 0.124 U/mg

0.223mg/ml Cat B = 98.9 U/mg COX = 38.906U/mg

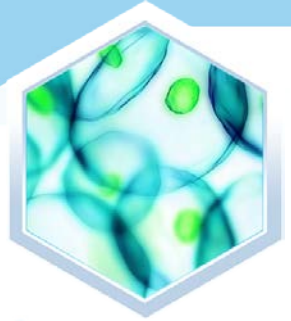
18.35mg/ml Cat B = 5.82 U/mg COX = .0047U/mg

Cat B units = 1 uM of AMC released / min
COX units = 1 uM of Cytochrome C oxidized / min

Conclusions



High specific activity for lysosomal enzymes (Acid phosphatase, cathepsin B, and nuclease) with minimal activity from enzymes associated with mitochondria.



Human Hepatic Lysosomes

Lysosome Isolation

Fresh human liver tissue

↓

Homogenization and low speed clarification (liver homogenate - LH)

↓

Pellet membranes by ultracentrifugation (crude lysosome fraction – CLF)

↓

Further purification of CLF by ultracentrifugation through Percoll gradient (heavy membranes – HM)

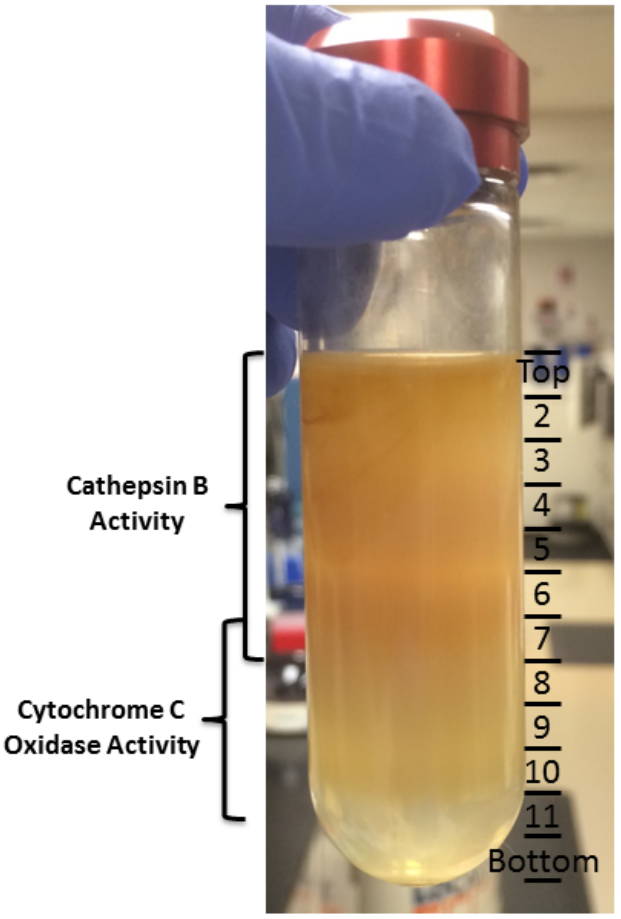
↓

Separation of Lysosomes from HM by ultracentrifugation through OptiPrep density gradient and fractionate into 12 equal factions (top to bottom)

↓

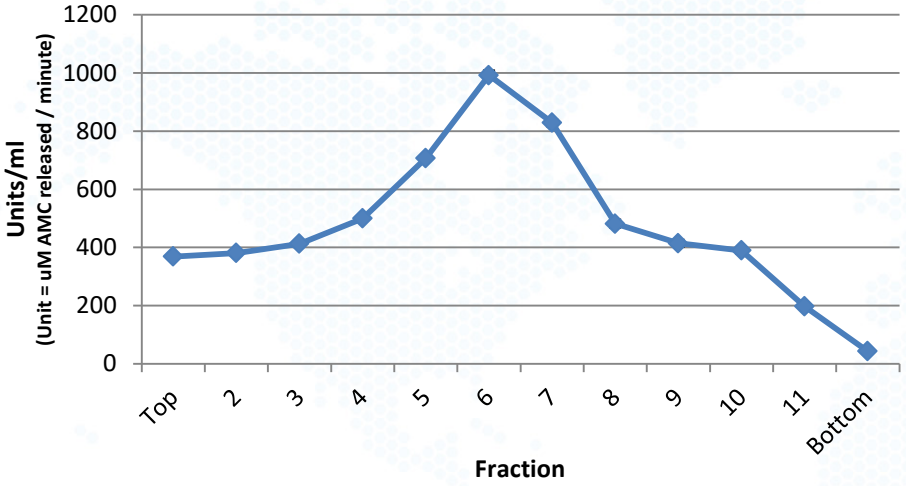
Characterization of lysosomal fractions by immunoblot, enzymatic activity, and protease content by immunoblot array.

OptiPrep Gradient

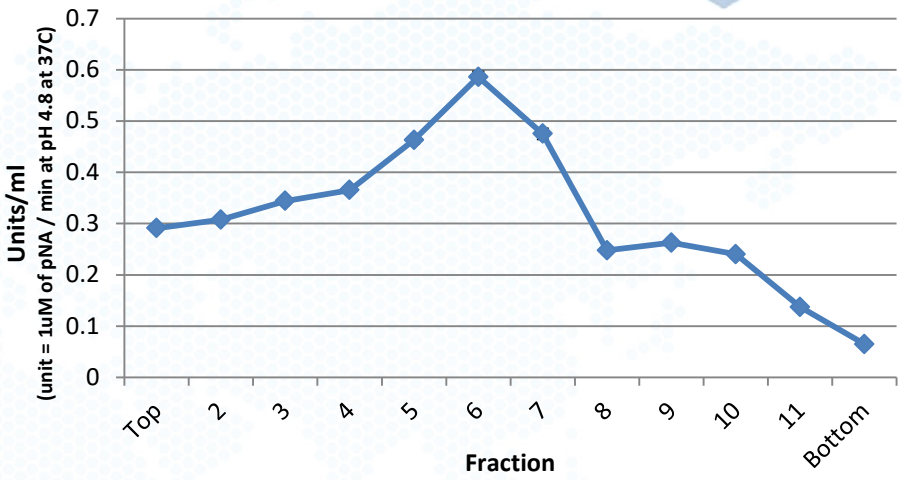


Identification of where the lysosome are in the gradient

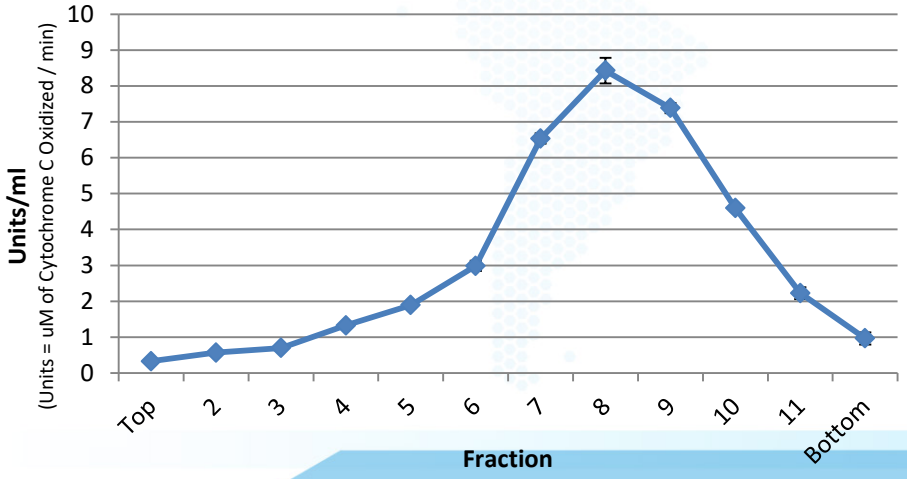
Cathepsin B



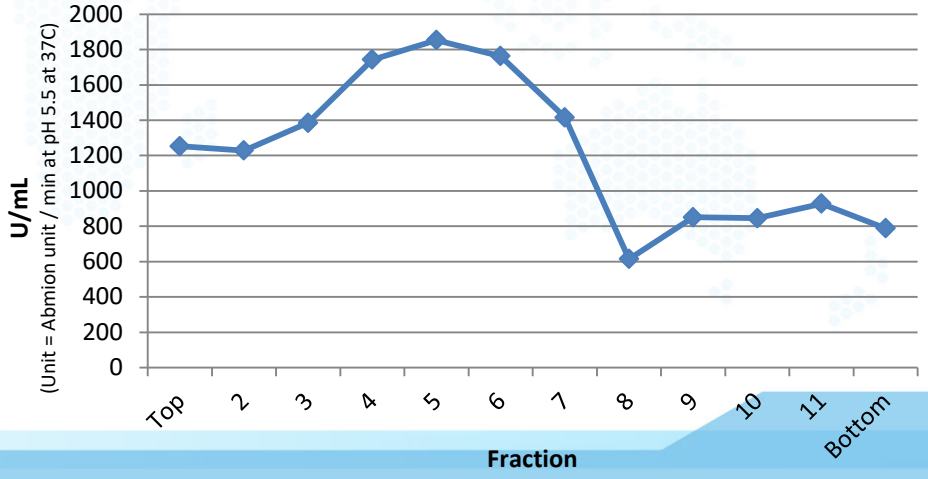
Acid Phosphatase



Cytochrome C Oxidase



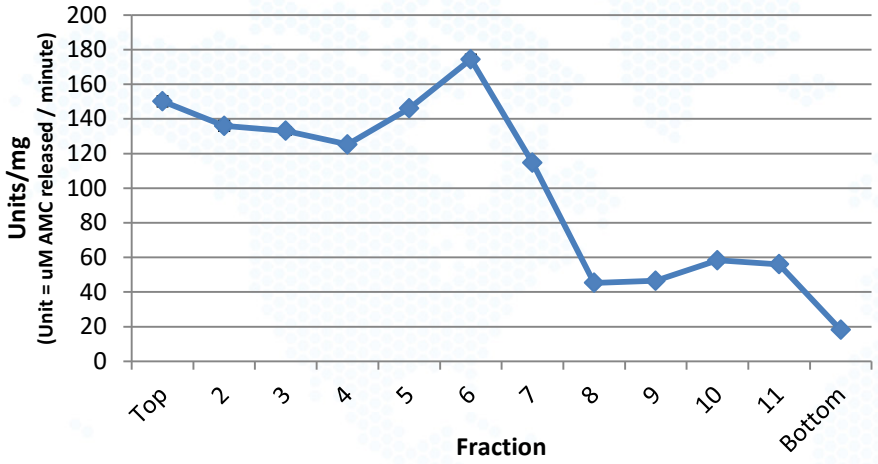
RNase



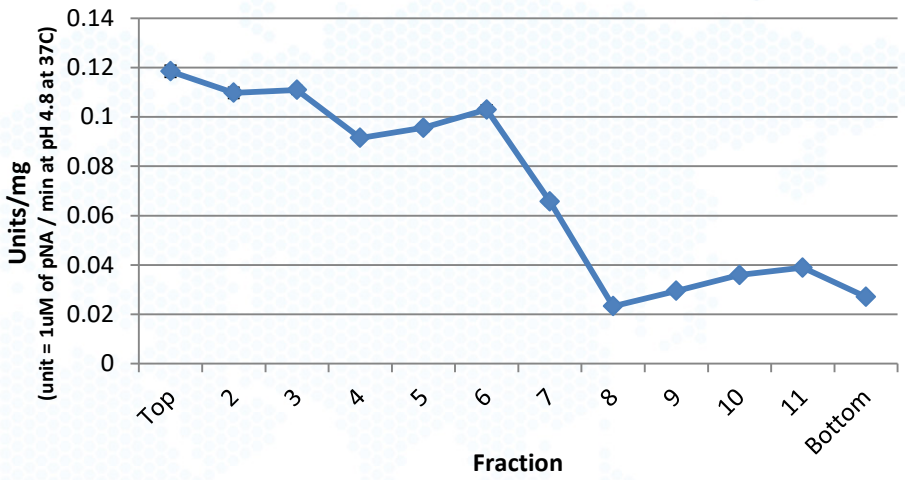
One unit is the amount of RNase A required to give an increase of 0.0146 A286 units per min in a 1 mL volume at RT and is equivalent to 0.1177 Kunitz Units.

Identification of where the lysosome are in the gradient

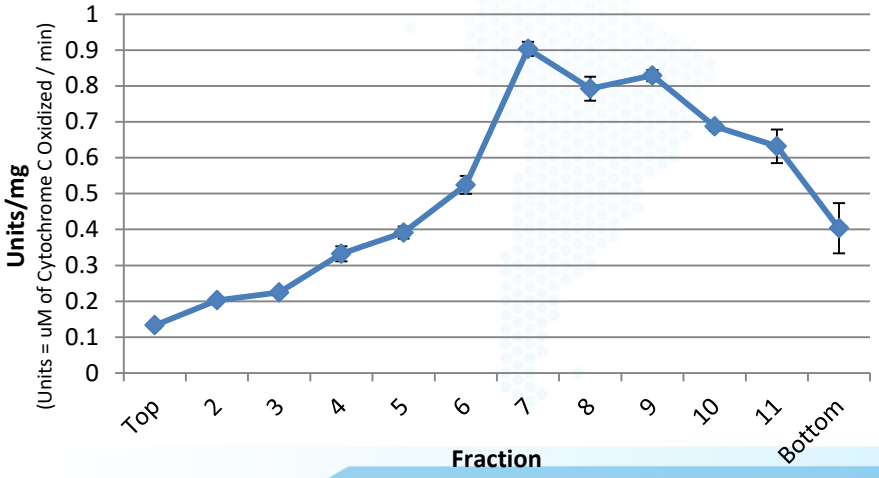
Cathepsin B



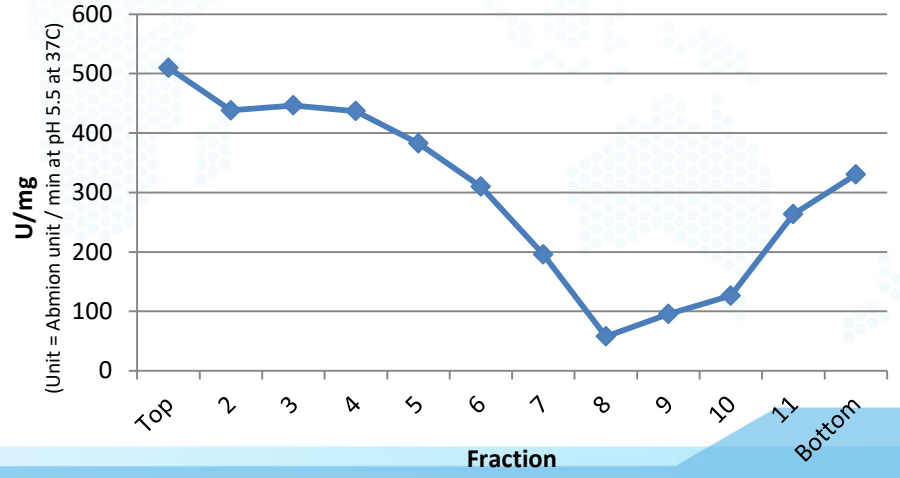
Acid Phosphatase



Cytochrome C Oxidase



RNase



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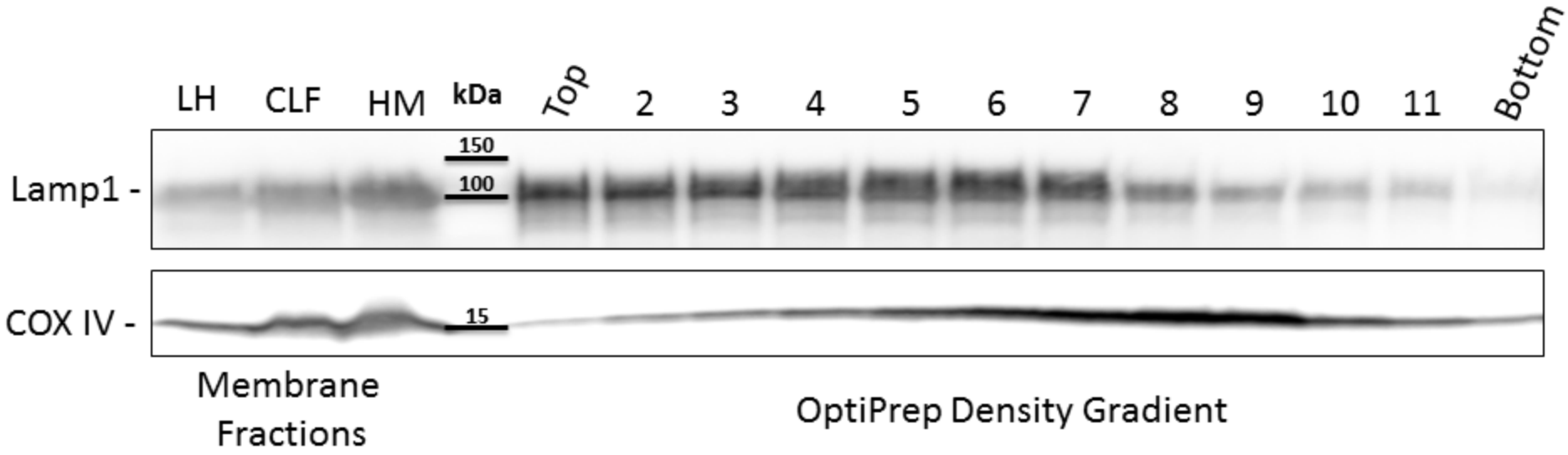
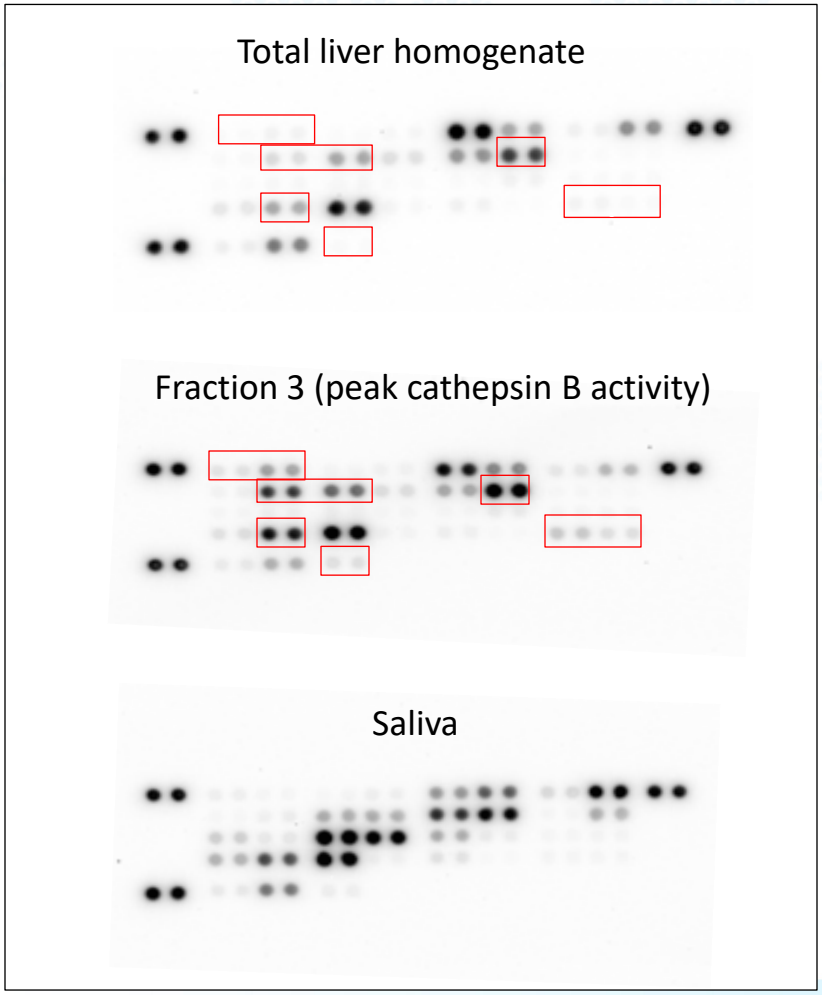
Identification of where the lysosome are in the gradient



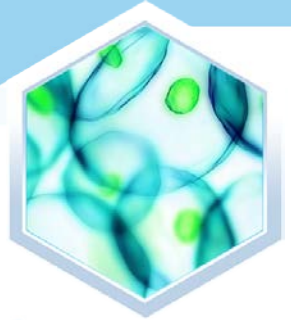
Proteases contained in fraction containing peak cathepsin

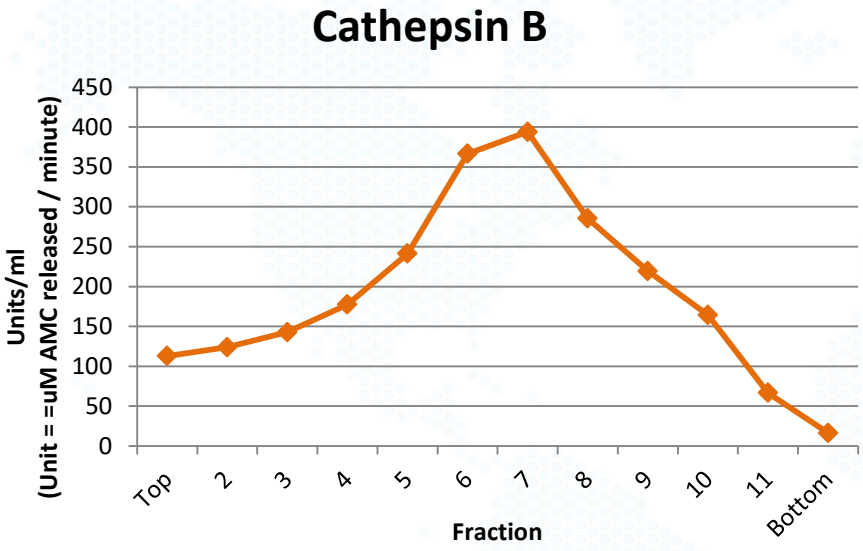


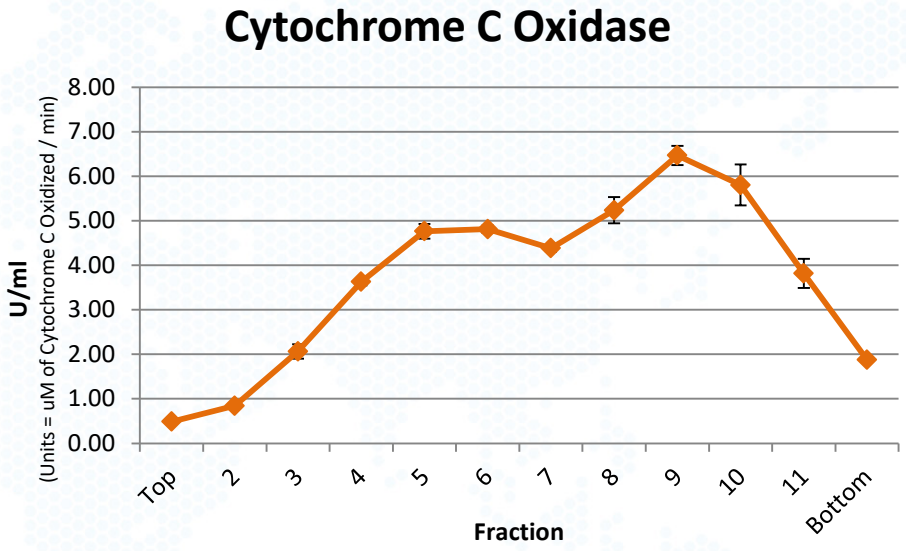
Cathepsin B-high activity fraction composed of disintegrin and metalloproteinase domain-containing proteins 8 and 9, cathepsins A, B, D, L, S and X/Z/P, dipeptidyl-peptidase 4, matrix metalloproteinases 8 and 9, neprilysin, presenilin and proteinase 3 (middle panel).

The gradient fractionation enriched cathepsins L and S, disintegrin and metalloproteinase domain-containing protein 8 and 9, dipeptidyl-peptidase 4, matrix metalloproteinase 8, Urokinase, neprilysin, and presenilin (boxed in red) but not the other proteases present in fraction 3.

Donor/tissue variation







We observed inter-donor variability in the separation of highest activities of the two enzymes

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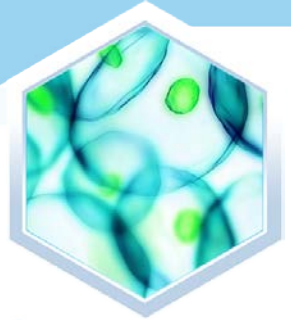
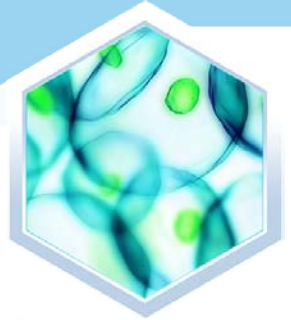


Conclusions

Purified human hepatic lysosomes show a 2-5 fold increase in lysosomal enzymatic activity per mg of total protein compared with initial liver homogenate.

Low contaminating activity from enzymes associated with mitochondria.

Serves as a *in vitro* reagent *with* a human matrix that compliments rat Tritosomes.



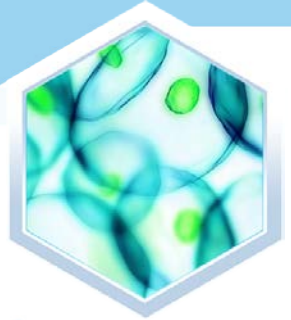
Further development of human hepatic lysosomes

Fractions 1 – 6 represent different densities, however they all contain significant amounts of Lamp1.

These fractions vary in density and likely vary in:

- enzymatic enzyme activity
- membrane composition
- Biological function

Are there different experimental conditions needed to asses catabolism with different classes of biopharmacuticals?



What are the current uses?

In vitro diagnostic tool to conveniently and quickly evaluate potential changes in lysosomal stability due to targeted modifications of the biopharmacutical/macromolecule during development

Help narrow and direct development tracks of biopharmacutical

- ADCs
- siRNA/RNAi
- Biodegradable copolymers
- Nonoparticles

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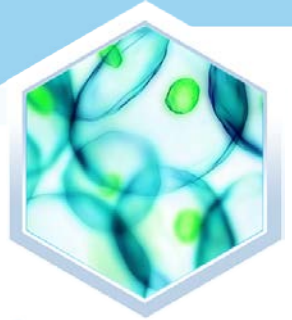


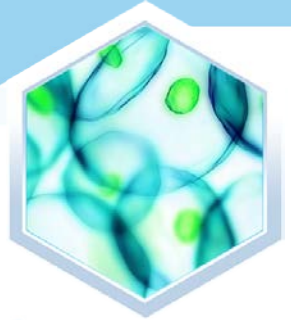
Available Product

H0610.L – Mixed Gender Human Liver Lysosomes, 0.25 mL

R0610.LT – Mixed Gender Rat Liver Tritosomes, 0.25 mL

Features & Benefits:

- Highly purified
- Characterized for lysosome specific enzymatic activity
- Less complex than *in vivo* models
- More representative than individually expressed/purified enzymes



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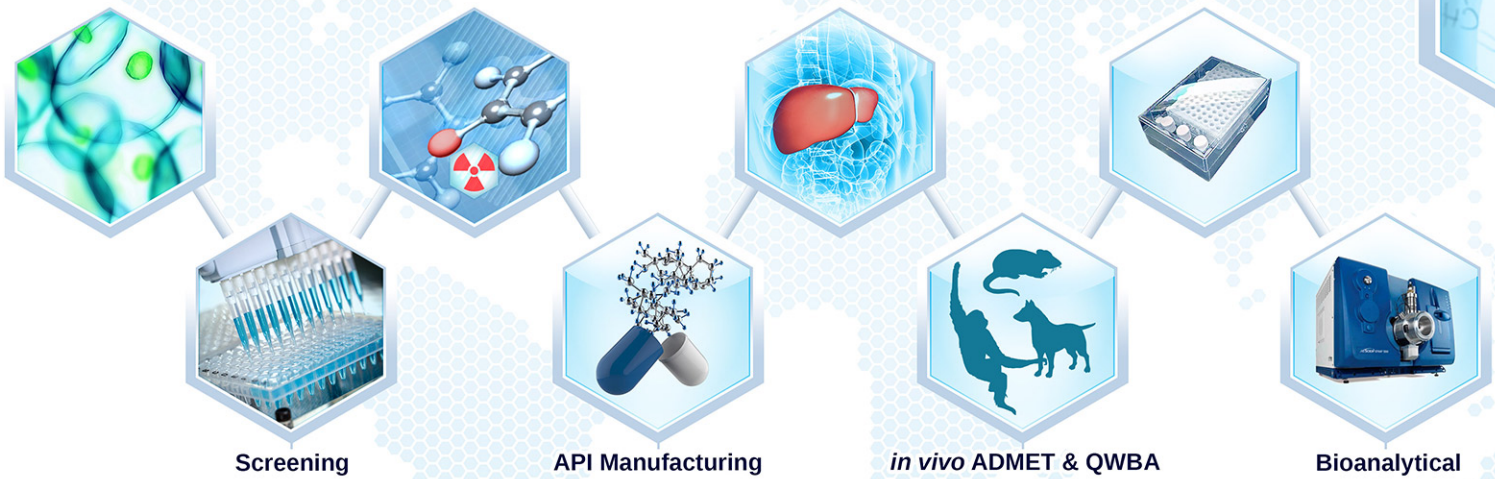

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