# Zymogram Preparation

# Zymogram

- Zymography is a modified protein electrophoresis technique.
- Use of electrophoresis to study enzyme activity.

- Zymography describes the experimental technique in which an enzyme activity is analyzed *in situ* following electrophoresis.
- It allows characterization of important physical properties of the enzyme within crude cell extracts or partially purified fractions.
- This can assist subsequent purification.
- Zymograms (also commonly known as “in-gel assays” or “activity gels”) have been used to analyze a wide variety of mammalian, plant, and microbial enzymes, including histone acetyltransferase, matrix metalloproteases, lipase, endoglucanase, phytase, Dnase, *α*- and an assortment of ribonucleases.

# Types

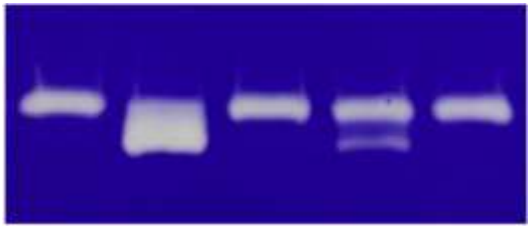
- Three types of zymography are used:
  - *in gel* zymography,
  - *in situ* zymography
  - *in vivo* zymography

# In-Gel Zymography

- In this modification of SDS-PAGE, the entire gel is polymerized with a protein substrate of interest, example gelatin or casein.
- Electrophoresis under nonreducing conditions and subsequent removal of SDS enable renaturation of the proteinase(s) in the sample, allowing for substrate degradation in the confines of the gel.
- This technique thereby elicits information on the estimated size of hydrplytic enzymes.
- Incubation of both sample and gel with class-specific enzyme inhibitors provides additional mechanistic depth.

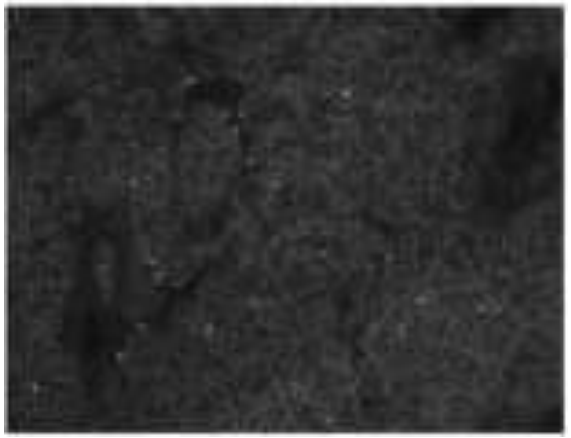
# Example: Gelatin zymography

- Incubate proteinase-containing sample(s) with nonreducing Laemmli sample dilution buffer (2.5% SDS) for 1 h on ice without boiling the sample(s). Ensure no contamination with beta-mercaptoethanol so that disulfide bonds remain intact.
- Prepare an 8%–9% SDS-polyacrylamide gel containing copolymerized gelatin or casein (0.1%). Carry out electrophoresis of sample(s).
- Wash gel 2 × for 20–30 min with 2.5% Triton X-100 to remove SDS and renature proteinase to restore enzymatic activity.
- Rinse gel with water 3 × to remove Triton X-100 prior to incubation for 24–48 h at 37°C in buffer of choice.
- After incubation, stain gels with Coomassie blue. Regions with proteolytic activity appear as clear regions against a blue background.



# In Situ Zymography

- In tissue sections, ISZ can recognise and localise specific protease activities.
- Frozen tissue sections can be incubated with a fluorescently labeled substrate to determine the localization of and qualitatively assess the abundance of a proteinase of interest.
- In situ zymography can also be used to assess gelatinolytic activity as shown in Figure.
- In this example, a section of an ovarian cancer xenograft tumor was overlaid with a quenched fluorescent gelatin (FITC-conjugated DQ-gelatin).
- Proteolytic cleavage of gelatin removes fluorescence quenching and enables visualization of the FITC signal.



# In-Vivo zymography (IVZ)

- IVZ allows for the detection of lysis of protein in a living organism.
- IVZ necessitates a biocompatible fluorogenic substrate that can be detected after breakdown due to lysis of protein.

# Advantages

- Zymograms have certain advantages over more conventional enzyme assays.
- Primarily, they allow enzyme activities to be attributed to polypeptides with defined physical characteristics, such as molecular weight or isoelectric point.
- In addition, it is possible using an in-gel assay to study heterogeneity of enzyme isoforms, multiplicity of enzymes (for example, in biological samples), and posttranslational modification of a particular enzyme (for example, glycosylation) and to simultaneously analyze an enzyme activity contained in various protein fractions.