A ß-Cell *Functional* Differentiation Diagnostic Laboratory - For Human Islets and Candidate Surrogate ß-Cells Alike

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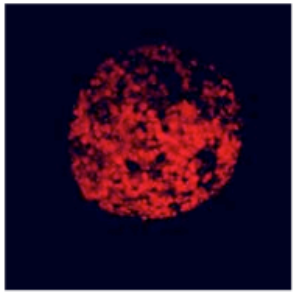
THE UNIVERSITY OF CHICAGO MEDICAL CENTER

The Pancreatic ß-Cell Functional Differentiation Analysis Laboratory



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JDRF Juvenile Diabetes Research Foundation International
dedicated to finding a cure

- **This laboratory provides a service to evaluate the *function* of candidate surrogate ß-cells (obtained from alternative sources including stem cells) and isolated human islets that may or may not have undergone various treatments.**
- **The following questions are addressed –**
  - ***Do the cells/islets synthesize proinsulin appropriately?***
  - ***If so, do the cells/islets convert proinsulin to insulin appropriately and efficiently?***
  - ***If so, do the cells/islets secrete insulin appropriately?***
- **HPLC and state of the art perifusion analyses are primarily used. Also, 'added extras' can be requested for further ß-cell functional characterization such as assessing unique ß-cell metabolic parameters, and real-time fluorescent based image analysis of secondary signal generation and exocytosis.**
- **How is it done? Either live candidate cells/islets can be shipped to the 'ß-Cell Functional Differentiation Analysis Laboratory' at the University of Chicago for analysis, or, by prior arrangement, we can consult with an investigator to conduct a protocol in their laboratory then cell/islet lysate samples subsequently shipped to Chicago for HPLC analysis.**
- **Confidentiality ensured. All data gathered for each analysis is held and treated in strict confidence, and only communicated to the investigator. Absolutely no data derived from this analysis will be published or publicly communicated without permission.**
- **Cost of the analysis will be partly subsidized but there will be some moderate charge-back costs to the investigator depending on the extent of the analysis requested. Please inquire prior to an analysis.**
- **For further information, questions and consultations please contact -**

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A ß-Cell *Functional* Differentiation Service Laboratory -

Goal: To provide a cost effective service for an unbiased functional diagnosis of human islet preparations, modified human islets, and candidate surrogate ß-cells.

The Questions -

Do the 'ß-cells' synthesize (pro)insulin appropriately

Do the 'ß-cells' secrete insulin appropriately

The Services -

Step - 1: Proinsulin synthesis/processing analysis -

Step - 2: Islet or 'ß-cell' perifusion analysis -

Step - 3: Optional extras -

Modest subsidized charge-back cost (reagents only).

ß-Cell Preparations to Diagnose

- **Isolated Human Islets**
- **'Insulin Positive Cells' derived from alternative sources**

  (e.g. **stem cells (embryonic or adult), liver, pancreatic exocrine cells, bone marrow, umbilical cord blood , gut cells, neurons, puppy-dog tails, extract of newt *etc.*)**

- **Human Islet ß-Cells undergone *in vitro* manipulations**

  (e.g. **mitogenic expansion, growth/survival factor treatment, gene therapy *etc.*)**

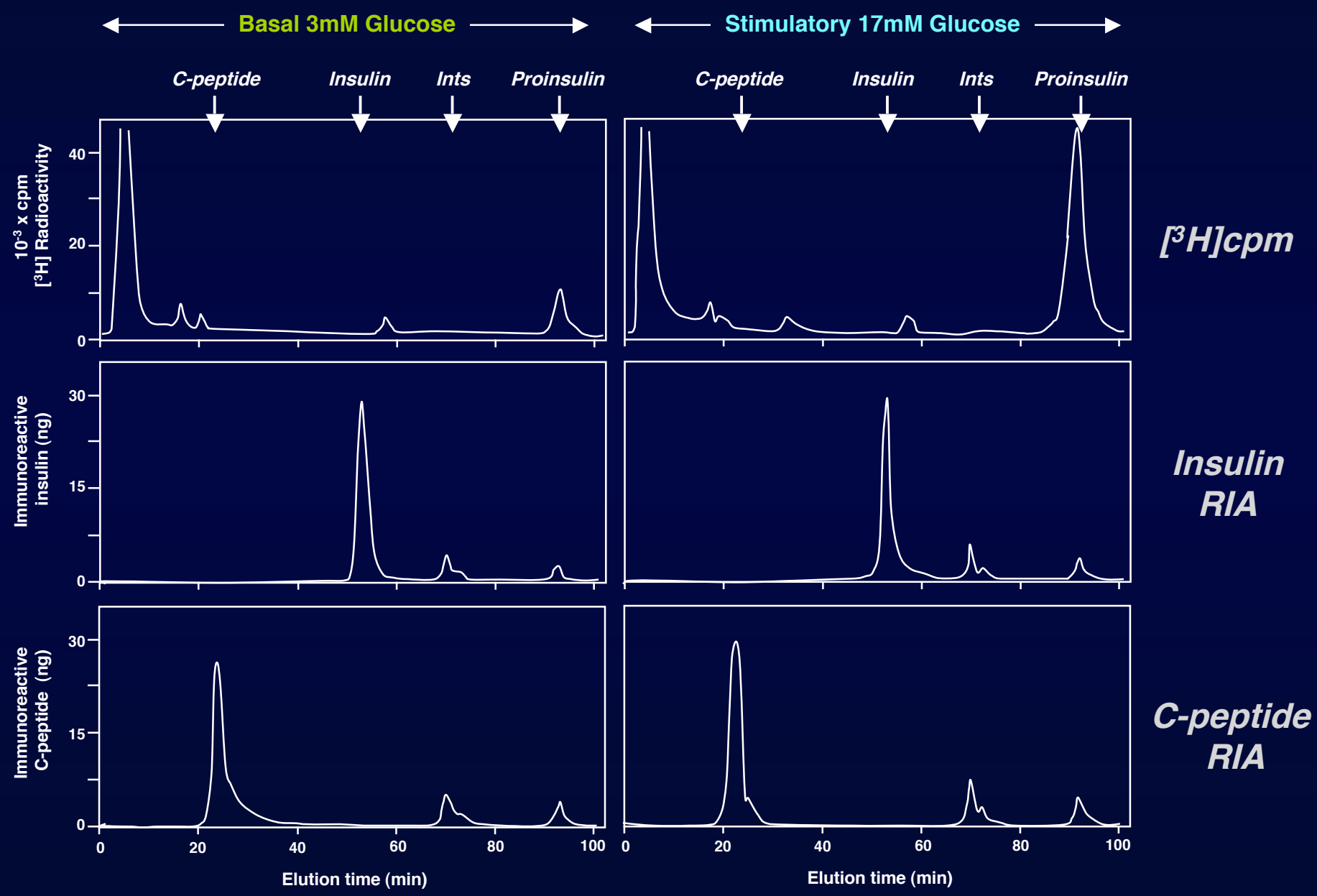
Step - 1a: Proinsulin biosynthesis analysis - Protocol

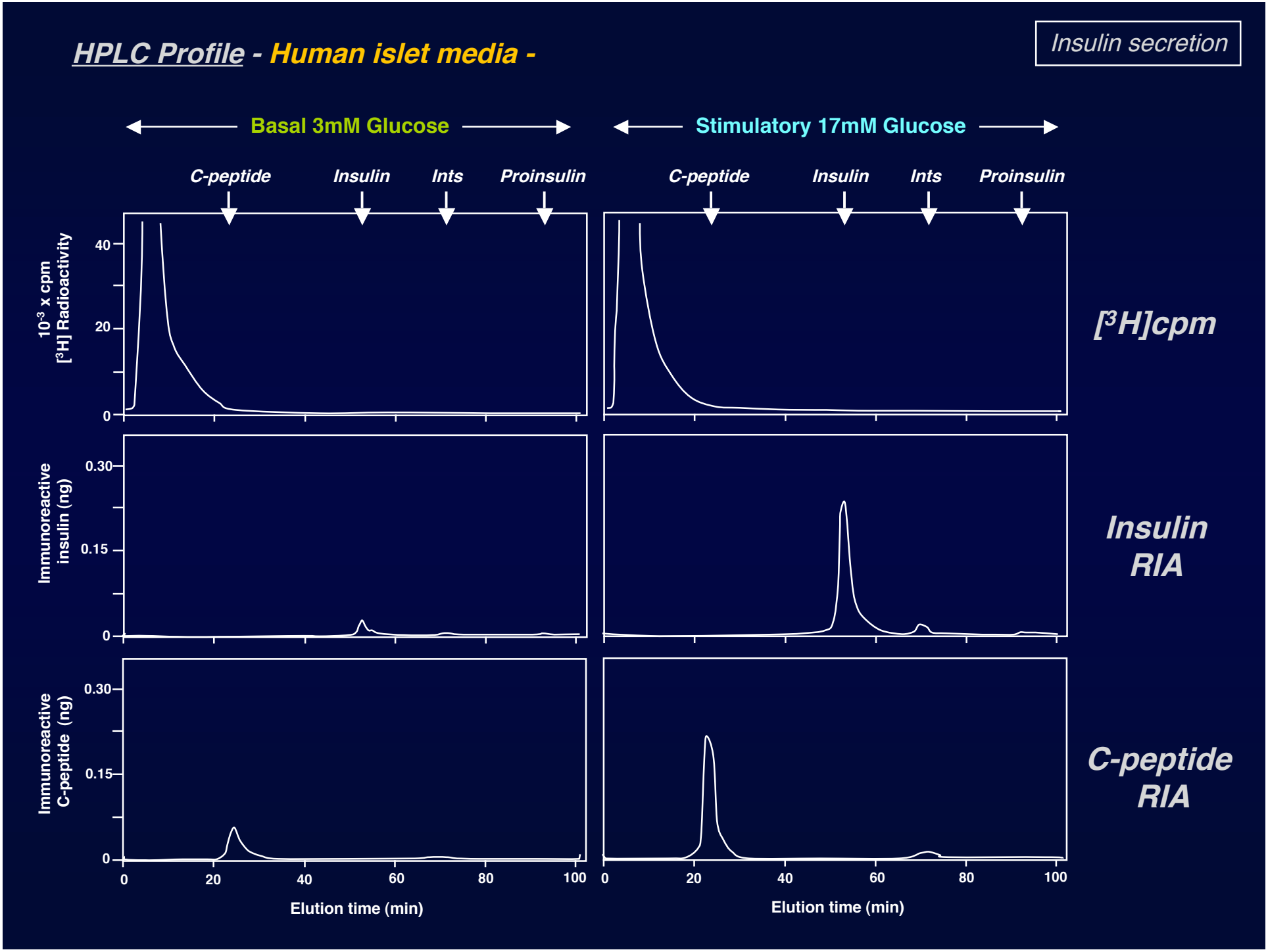
- 50-100 human islets or $5x10^5$ 'candidate beta-cells' per incubation
- Pulse radiolabeling -
  - 90 min preincubation at basal (3mM) glucose, then -
  - 60 min incubation at basal (3mM) or stimulatory (15mM) glucose, the -
  - last 20 min + [^{3}H]leucine
- Collect islets/cells and media (acid extract islets/cells)
- HPLC analysis

→ cpm and RIA (insulin and C-peptide) of fractions

HPLC Profile - Human islet extracts -

Proinsulin Synthesis




HPLC Profile - Human islet media -
Insulin secretion
Basal 3mM Glucose
Stimulatory 17mM Glucose
C-peptide
Insulin
Ints
Proinsulin
C-peptide
Insulin
Ints
Proinsulin
10-3 x cpm
[3H] Radioactivity
40
20
0
[3H]cpm
Immunoreactive
insulin (ng)
0.30
0.15
0
Insulin
RIA
Immunoreactive
C-peptide (ng)
0.30
0.15
0
C-peptide
RIA
0
20
40
60
80
100
Elution time (min)
0
20
40
60
80
100
Elution time (min)

Information That Can Be Derived From HPLC Analysis of Glucose Regulated Proinsulin Biosynthesis in Human Islets

Islet extract :

- Glucose regulated proinsulin biosynthesis
- Cell viability ([^{3}H]-leucine uptake)
- General protein synthesis
- Insulin content -
  - Proinsulin:Insulin ratio
  - Insulin:C-peptide ratio

Islet incubation media :

- Glucose stimulated insulin and C-peptide secretion
- Percent of islet insulin/C-peptide stores secreted
- Secreted Insulin:C-peptide ratio
- Basal proinsulin, insulin and C-peptide secretion
- 'Leakage' of [^{3}H]proinsulin - cell viability

NOTE - Time for glucose regulated [^{3}H]proinsulin biosynthesis analysis ~8h total
Full analysis is 24-36h

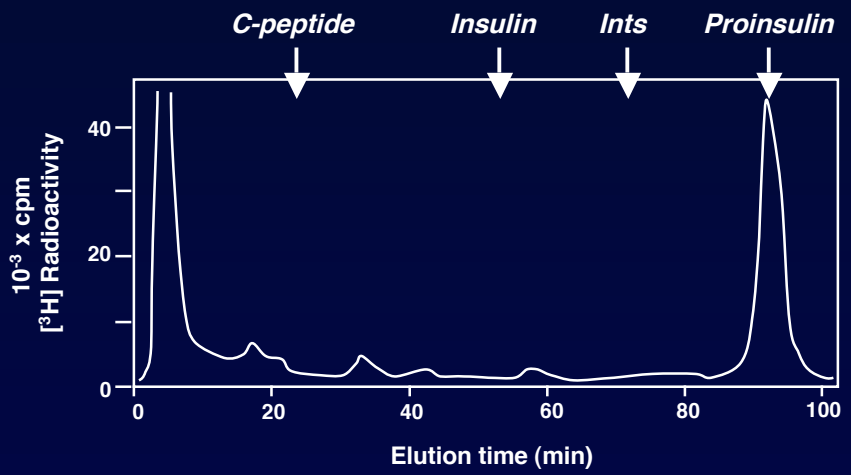
Step - 1b: Proinsulin processing analysis - Protocol

- 100-200 human islets or $8x10^5$ candidate beta-cells per incubation
- Pulse-chase radiolabeling -
    - 90 min preincubation at basal (3mM) glucose, then -
    - 60 min incubation at stimulatory (15mM) glucose (last 20 min + [^{3}H]leucine - 'pulse')
    - 90 min 'chase' at basal (3mM) or stimulatory (15mM) glucose
- Collect islets/cells and media (acid extract islets/cells)
- HPLC analysis

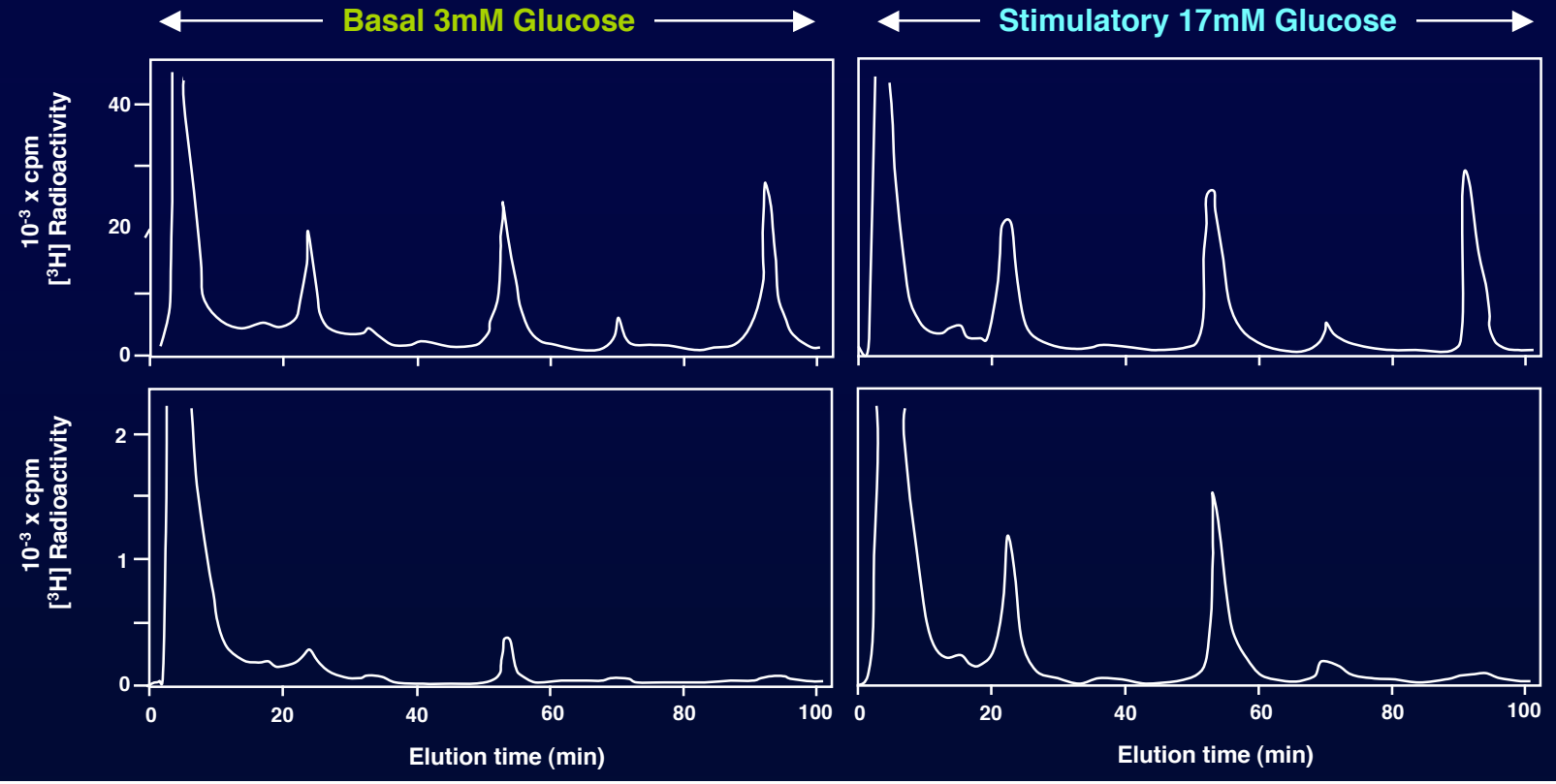
→ cpm and RIA (insulin and C-peptide) of fractions

HPLC Profile - Human islet extracts/media -

[3H]Proinsulin processing
[3H]Insulin/C-peptide secretion



ISLET S-
30 min pulse [3H]Proinsulin

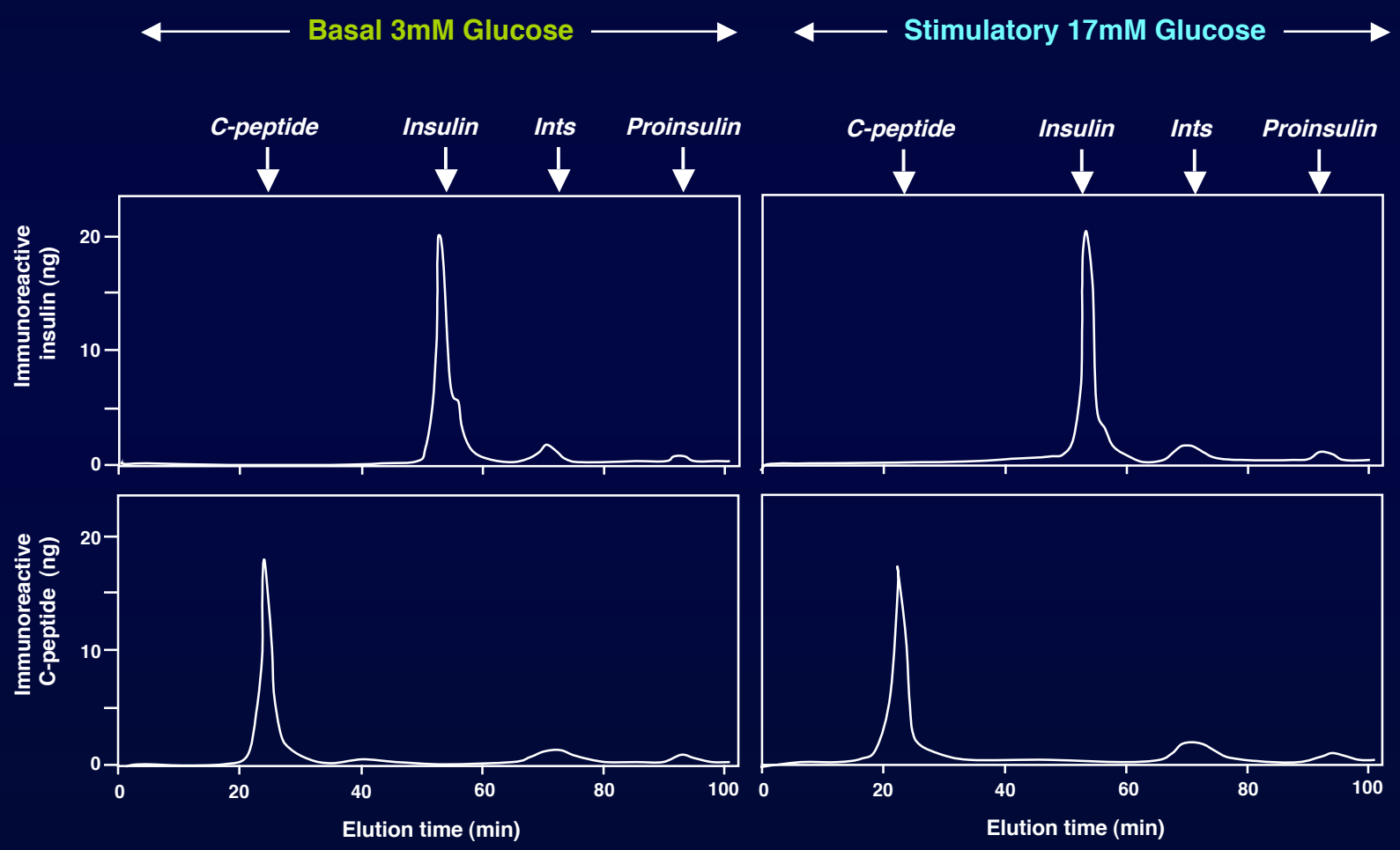


ISLET S-
90 min Chase
[3H](Pro)insulin

MEDIA-
90 min Chase
[3H](Pro)insulin

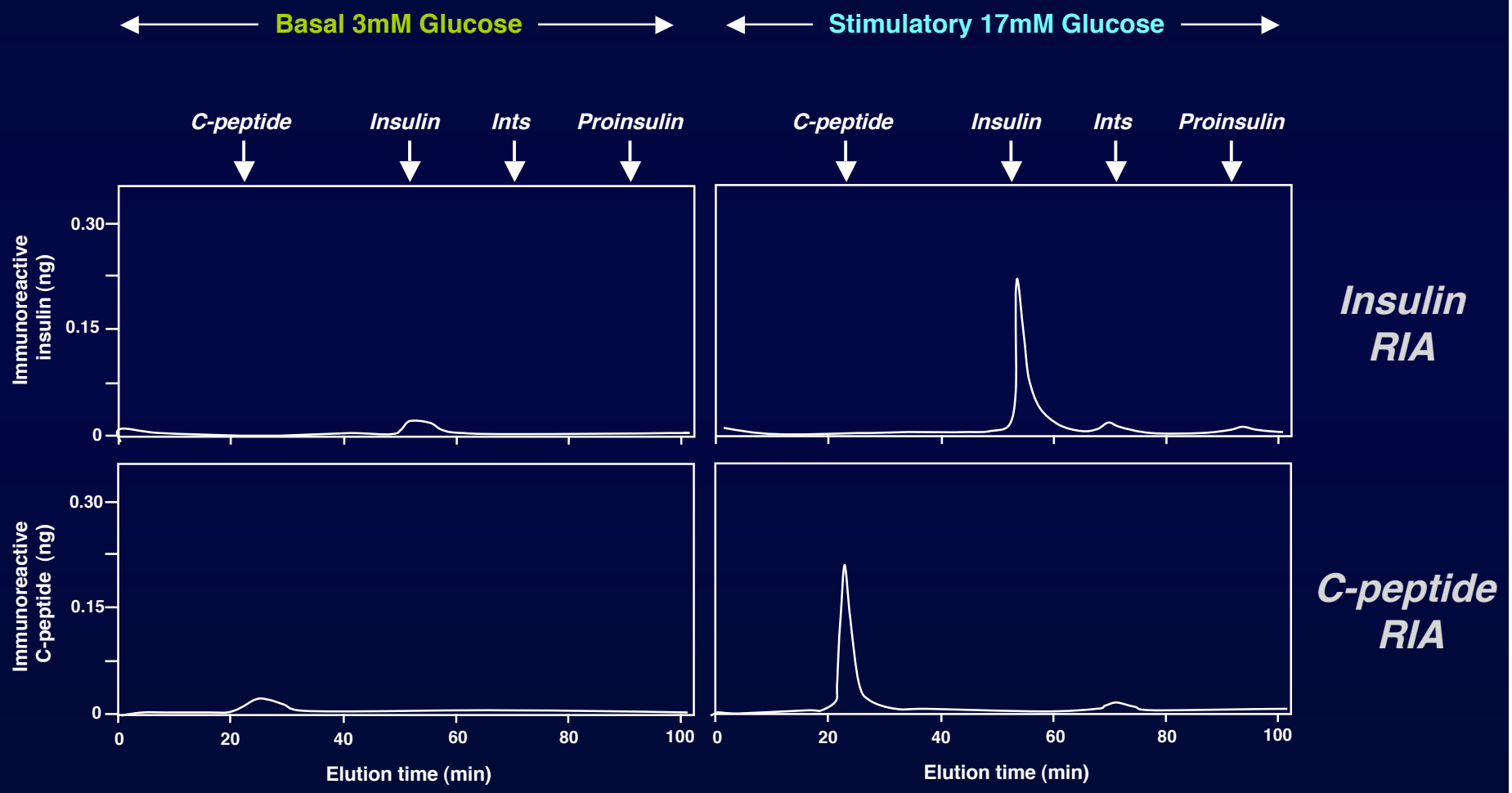
Insulin/C-peptide content

HPLC Profile - Human islet extracts -



Insulin/C-peptide secretion

HPLC Profile - Human islet media -



Information That Can Be Derived From HPLC Analysis of Proinsulin Processing in Human Islets

Islet extract :

- **Proinsulin biosynthesis (but not translational regulation by glucose)**
- **Cell viability ([^{3}H]-leucine uptake)**
- **General protein synthesis**
- ***Proinsulin processing efficiency***
- **Insulin content -**
  - **Proinsulin:Insulin ratio**
  - **Insulin:C-peptide ratio**

Islet incubation media :

- **Glucose stimulated insulin and C-peptide secretion**
- **Percent of islet insulin/C-peptide stores secreted**
- ***Preferential secretion of newly synthesized Insulin/C-peptide***
- **Secreted Insulin:C-peptide ratio**
- **Basal proinsulin, insulin and C-peptide secretion**
- ***Unregulated [^{3}H]proinsulin secretion***

If Step 1 looks good, go on to Step 2 ……

Step - 2: Islet or ‘candidate ß-cell’ perifusion analysis -

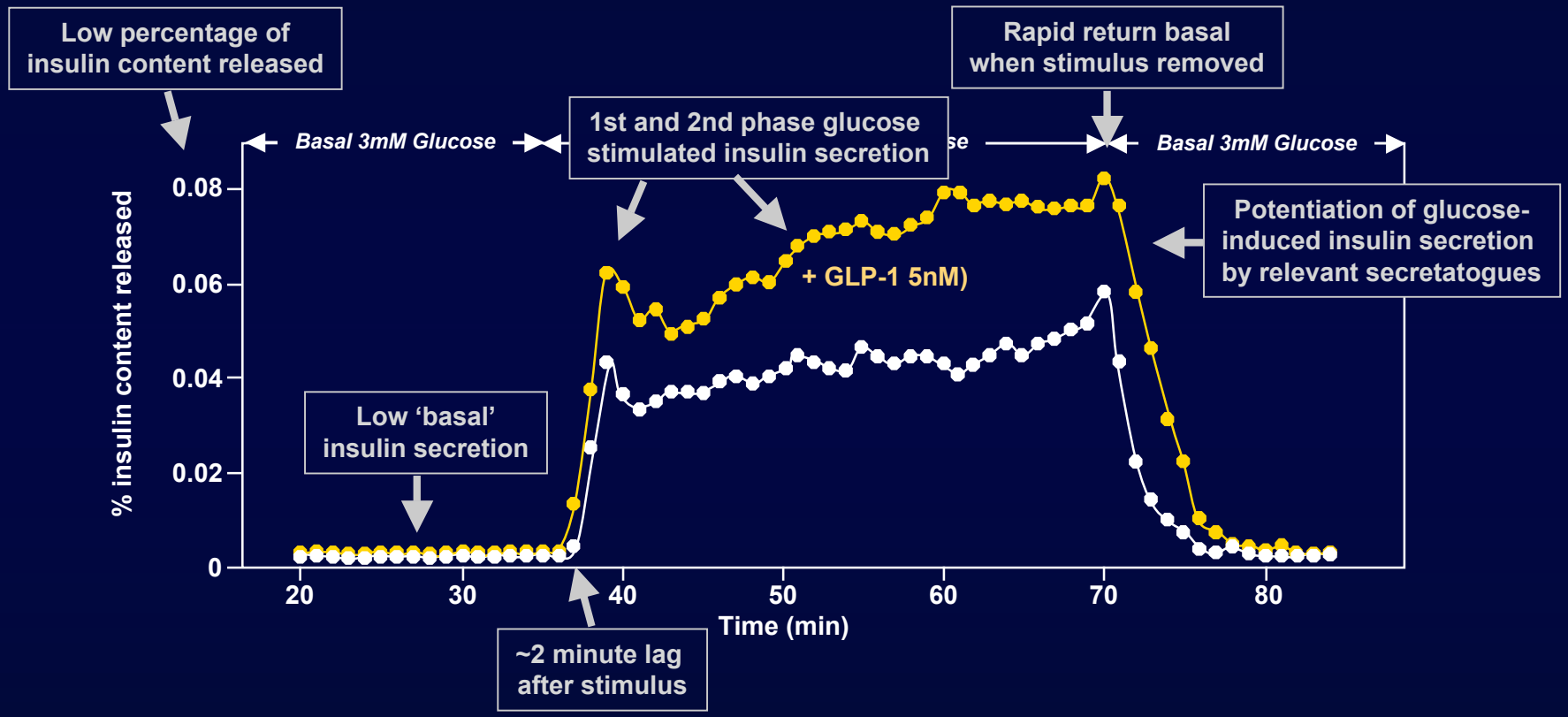
- 50-100 human islets or $5x10^5$ ‘candidate beta-cells’
- Perifusion apparatus critical components -

ISLET CHAMBER
Small Swinnex filter holder
with glass fiber filter

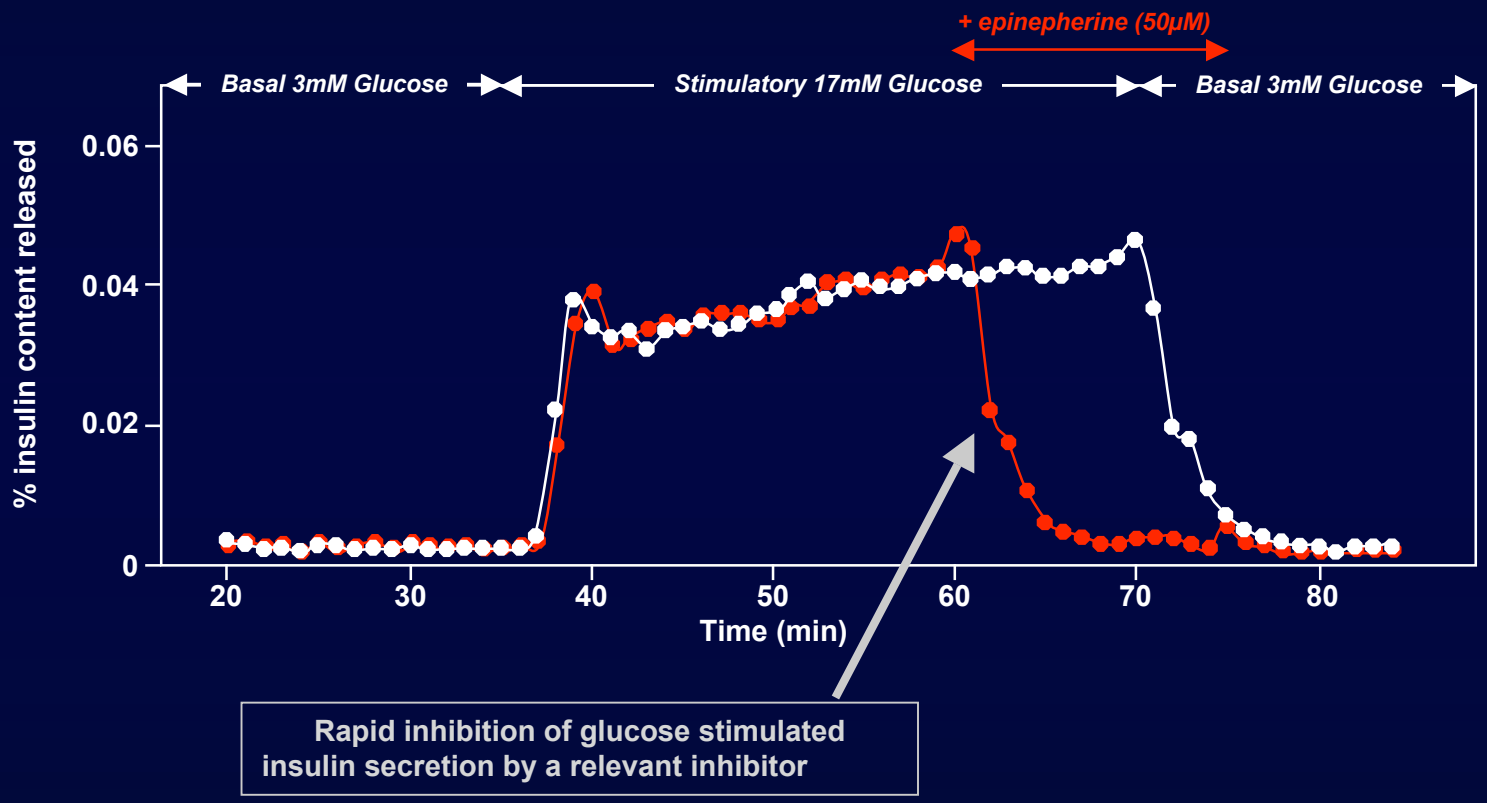
HIGH QUALITY TUBING PUMP
(ISMATEC)

RELIABLE FRACTION COLLECTOR

Step - 2: Human islet perifusion profile - the ‘on switch’ protocol



Step - 2: Human islet perifusion profile - the ‘off switch’ protocol

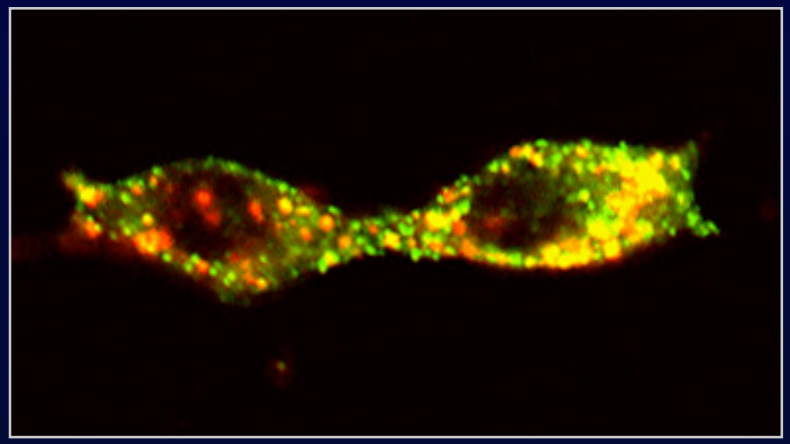


If Steps 1 & 2 look good ……

is any extra analysis needed?

Step - 3: Optional extras -

- Metabolic parameters
- Secondary messengers
- Insulin secretory trafficking/exocytosis
- Confocal and EM analysis




THANK YOU