

EXPERIMENTAL PLAN

Follicular Cell Safety Gate

Purpose

Exclude formulations that impair follicular cell viability or induce stress.

Model

- Human dermal papilla cells (hDPCs)

Assays

1. Cell viability

MTT or Resazurin

Exposure: 24 h and 72 h

2. Cell stress

ROS generation (DCFDA)

Parameters

- Acceptance criteria: $\geq 90\%$ viability vs vehicle

No ROS increase $> 20\%$ vs vehicle

Decision

- Fail = terminate hair-growth claim or reformulate
- Pass = proceed to mechanistic assays

Growth Suppression Risk Screening

Purpose:

Confirm the formulation does **not activate catagen-promoting pathways**.

Assays

1. TGF- β 1-induced Smad2/3 phosphorylation

Western blot or ELISA

2. DKK1 expression

qPCR or ELISA

Interpretation

- Desired: \downarrow Smad2/3 activation \downarrow DKK1 expression
- Neutral effect acceptable
- Increase = high-risk signal

Stop rule

- Upregulation of catagen markers = abandon growth positioning