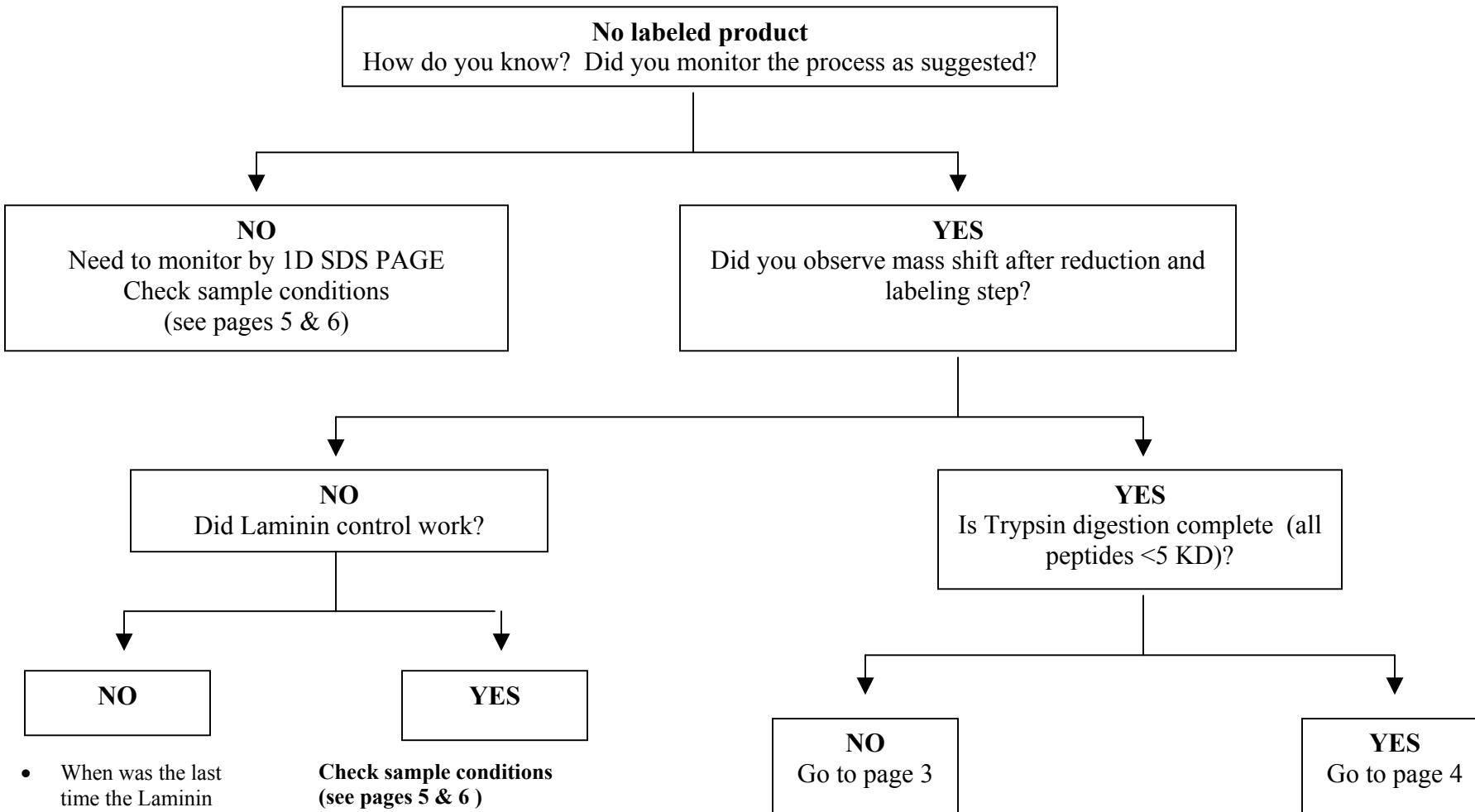


Cleavable ICAT[®] Reagent Kit Troubleshooting

Problem	Possible solution(s)
Saw ICAT [®] Reagent pairs, but database search did not come up with any hits	<ul style="list-style-type: none"> • Check cleavage conditions (Biotin tag may still be on the peptide). See pages 5 and 6 below • Check Mass Spec accuracy • Check software search parameters
Saw ICAT [®] Reagent pairs, but intensity is lower than expected	<ul style="list-style-type: none"> • Remove any high abundance proteins which may be masking the presence of low levels of ICAT[®] Reagent pairs • Do a protein assay to establish the actual concentration of protein in your samples (actual protein concentration may be lower than expected) • See pages 5 and 6 below for the proper conditions at each step • Spike with known peptides for sensitivity check
No ICAT [®] Reagent pairs seen	<ul style="list-style-type: none"> • Protein may contain no cysteines • Follow the troubleshooting trees on the following pages • Did you centrifuge the reagent tubes and can you see the reagent on the bottom of the tubes?

Cleavable ICAT® Reagent Kit Troubleshooting Tree



- When was the last time the Laminin control worked?
- What has changed since then?
- Was the kit stored properly?
- Did you use the buffers supplied in the kit or did you make your own?

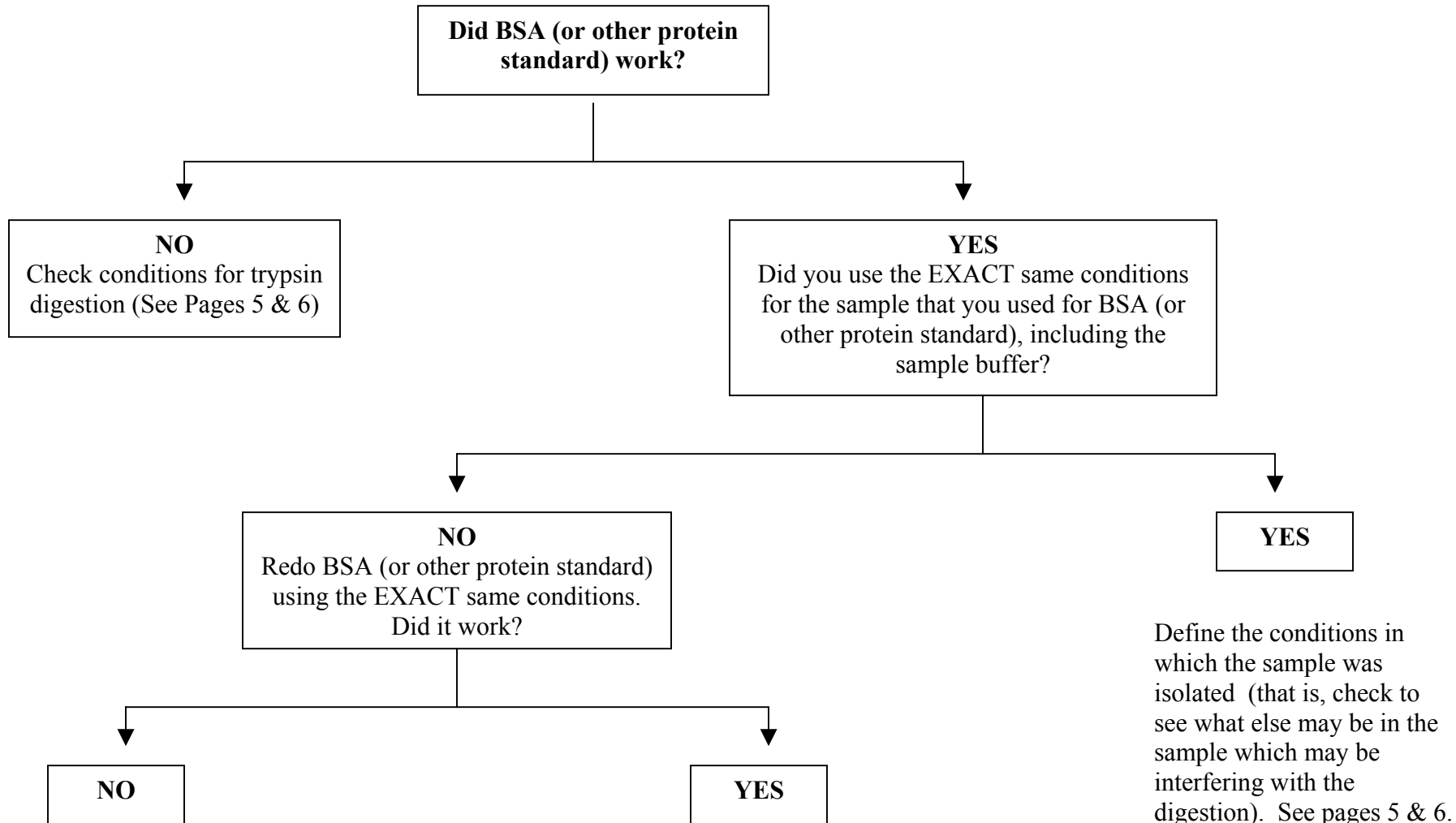
**Check sample conditions
(see pages 5 & 6)**

- Concentration and type of detergent
- Concentration of salt
- pH
- Sample concentration
- ICAT® Reagent concentration

NOTE: if conc. of sample or ICAT® reagent is too low, efficiency will be low

For Reference only

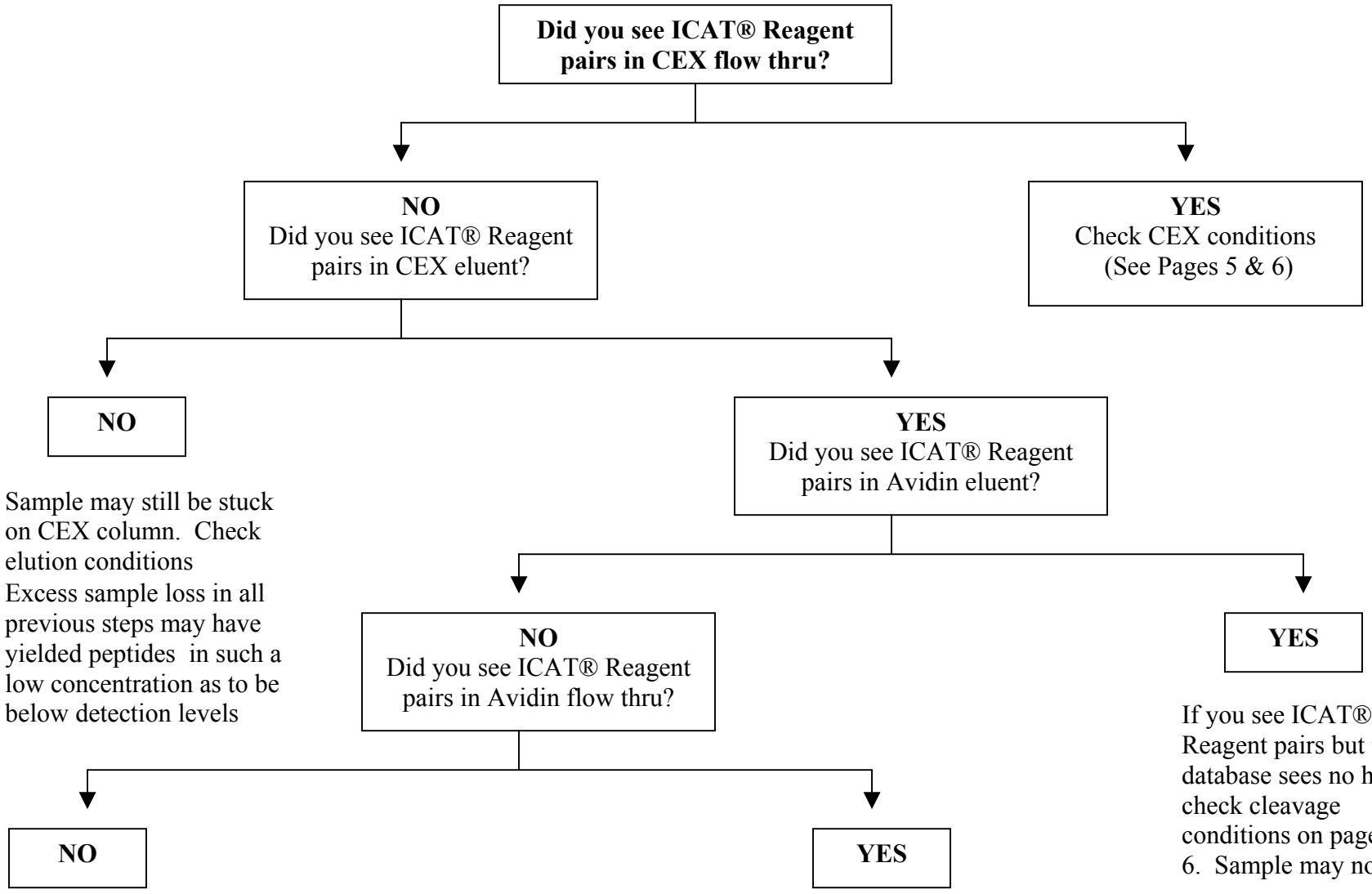
Trypsin digestion is NOT complete/did not work (no mass shift after digestion)



Modify the conditions until BSA (or other protein) works, such as diluting sample, buffer exchange, or acetone precipitation

For Reference only

**Trypsin digestion is complete
All Masses < 5KD**



- May have very little sample. Concentrate the sample

Proper conditions for Cleavable ICAT® Reagent Labeling Kit

STEPS	pH	Salt Conditions	Acceptable (Buffer) Components/concentrations	Things to avoid	Comments
Reduction	8.5		<ul style="list-style-type: none"> • TCEP, pH adjusted (7-7.5) at 1 mM • TBP at <u>5</u> mM <p style="text-align: center;">Either at 100°C for 10 minutes</p> <ul style="list-style-type: none"> • Urea: 37-50°C for 20-30 min 	DTT: interferes with labeling	Protein conc. and ICAT® Reagent conc. can effect efficiency; lower concentrations will reduce efficiency. Control concentration of protein is approximately 1.2 mg/ml
Labeling	8.5	≤ 50 mM	0.1% SDS 2% octylglucopyranoside, 2% Triton X-100 2% NP-40 2% Tween 20 2% CHAPS Fresh 8M urea	Thiourea	<ol style="list-style-type: none"> 1. To remove unwanted contaminants, do acetone precipitation 2. Labeling solution: 1.75 mM
Digestion	8.5		0.05% SDS 1% octylglucopyranoside 1% Triton X-100 1% NP-40 1% Tween 20 1% CHAPS 1M urea	ACTIVE Protease inhibitors	10-25 mM Tris pH 8.5 with 5-10mM CaCl ₂ will enhance efficiency of Trypsin
CEX	3.0	≤10 mM		Guanidine HCl	pH of 2.5 to 3.3 is acceptable. DO NOT go lower or higher. Use phosphoric acid to adjust the pH.

STEPS	pH	Salt Conditions	Acceptable (Buffer) Components/concentrations	Things to avoid	Comments
Avidin	7.2				<ol style="list-style-type: none"> 1. MUST activate cartridge 2. Use 2 x PBS to adjust before loading onto avidin column 3. DO NOT OVERLOAD: capacity is 8-10 nmol 4. MUST wash to lower salt concentration and remove non-specifically bound peptides (Wash 2: Biocarb/MeOH pH 8.3) 5. DRY completely
Cleavage		Must be salt FREE		Salts, water	DRY COMPLETELY