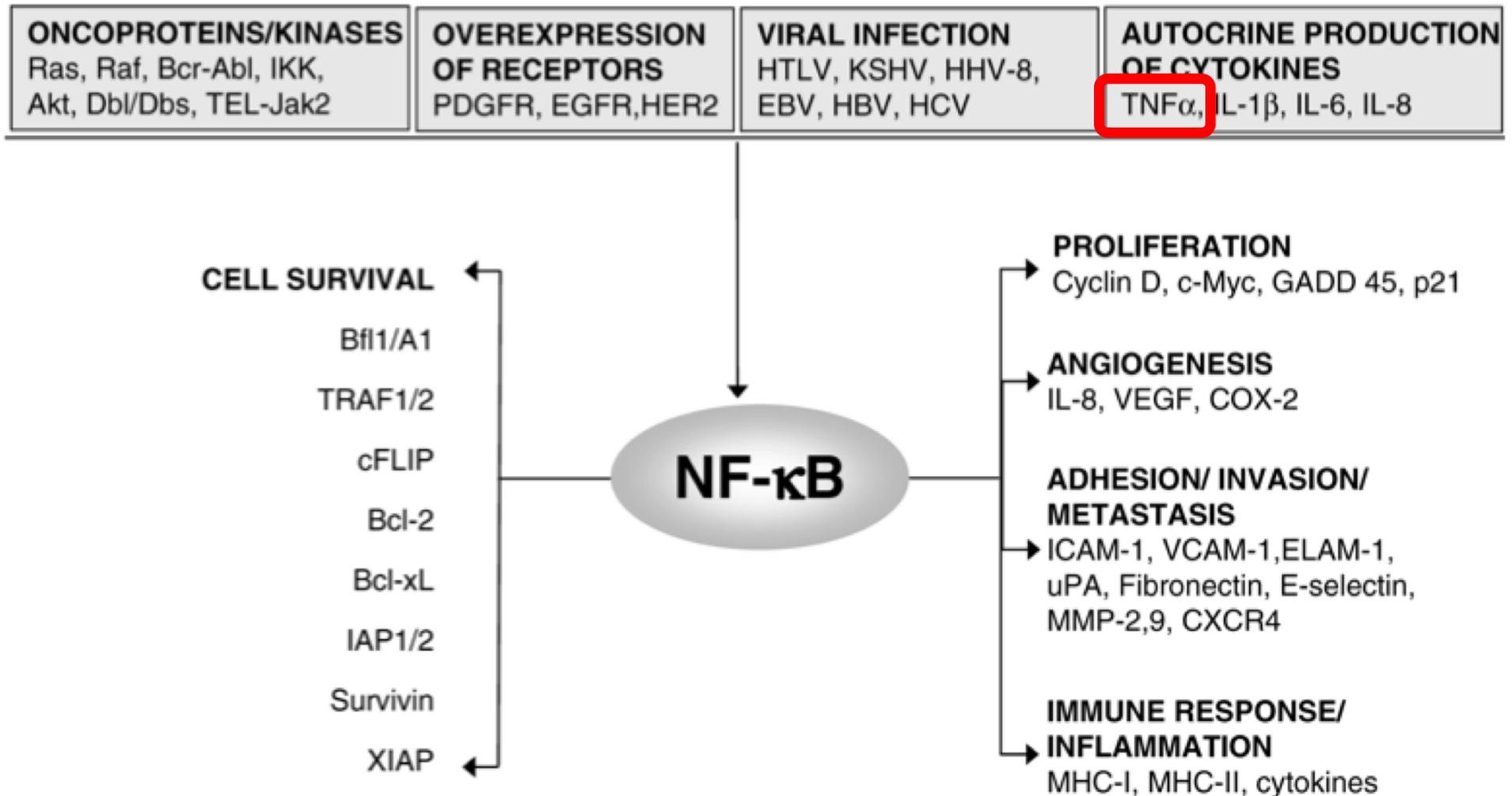


Bioquímica Experimental IV

2017 – 2018

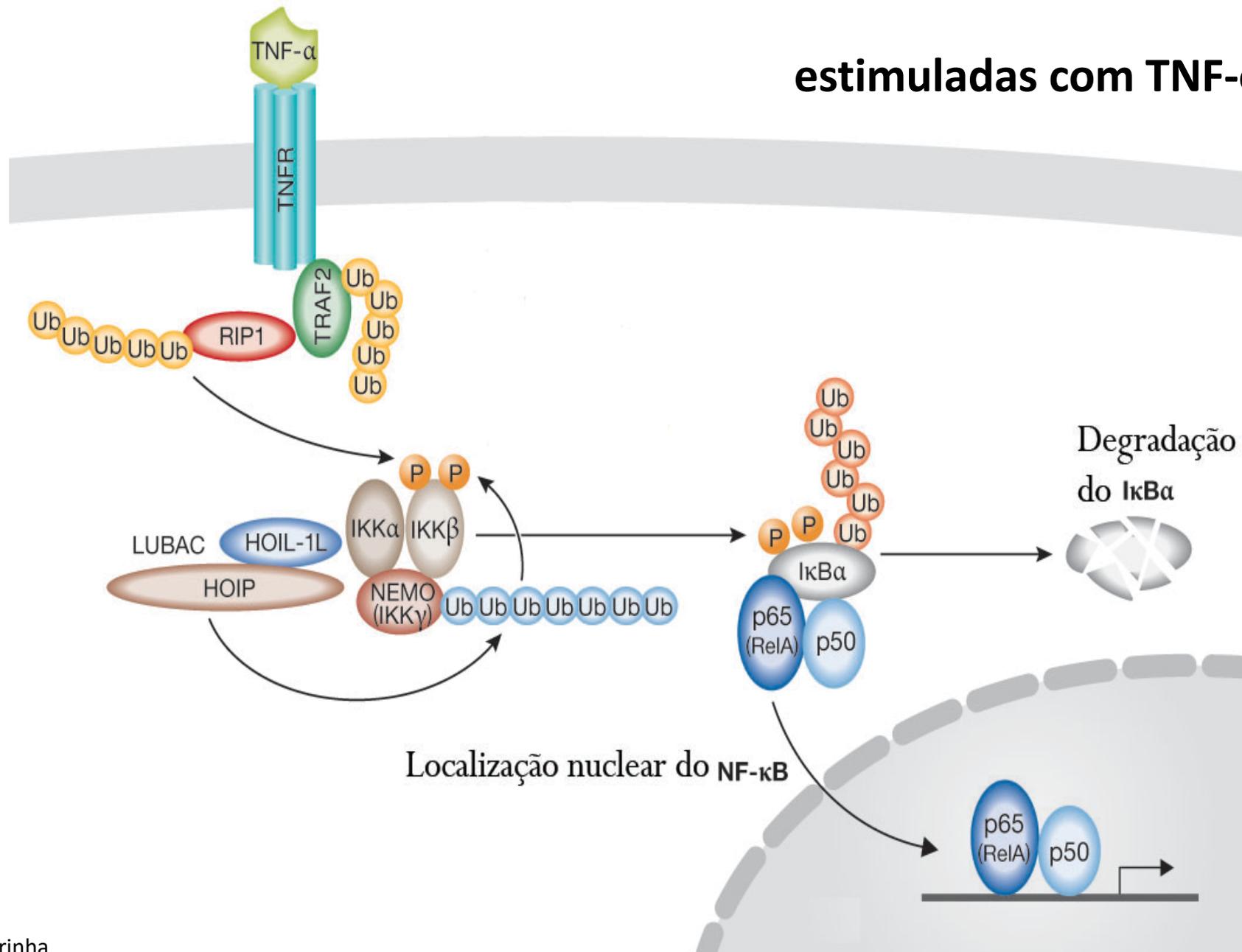
Western blotting/Immunoblotting

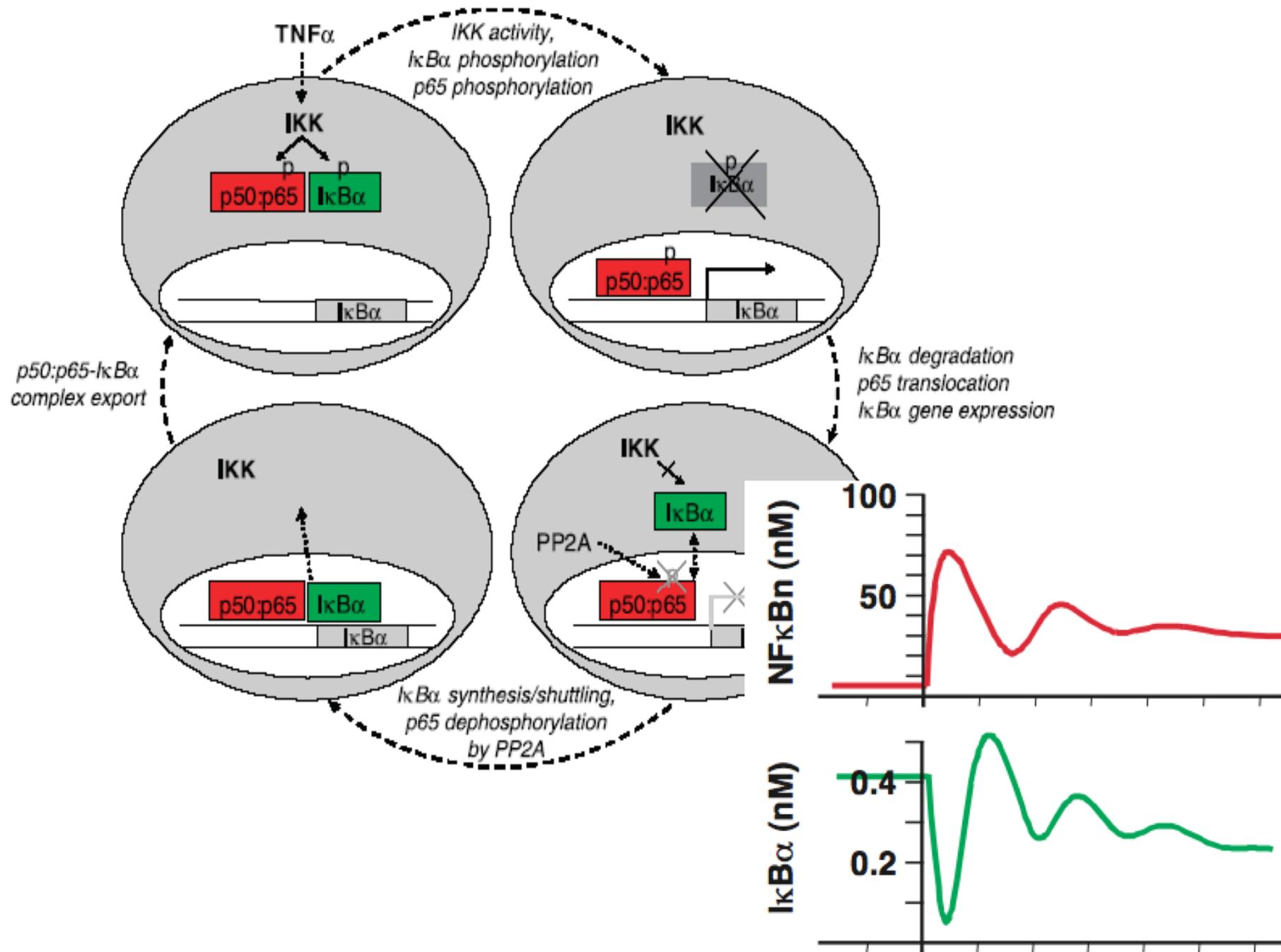


MECHANISMS OF CONSTITUTIVE ACTIVATION OF NF- κ B

Dinâmica da Localização do Factor NF- κ B em células HeLa

estimuladas com TNF- α





Dia 1

Extracção diferencial de proteínas do citosol e do núcleo

- Células HeLa
- Tratamento com TNF- α , com e sem inibidor do proteassoma (MG-132).
- Lise das células (membrana plasmática) a vários tempos (tampão contendo NP-40)
- Sedimentação dos núcleos
- Lise da membrana nuclear (elevada força iónica)
- Quantificação da concentração de proteína total nas amostras

Dia 2

Western blot das fracções nuclear e citosólica SDS-PAGE e Transferência



Sample preparation

Lysis of sample in appropriate lysis buffer

Protein assay to determine protein concentration

Add loading buffer, heat at 100°C 10min.



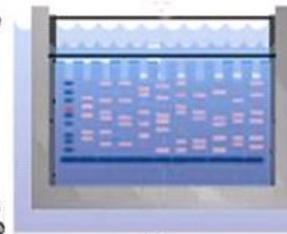
Loading the gel

Optimize loading amount according to the expression level of the protein

Running the gel

Successful separation of proteins through the gel due to different molecular sizes; the smaller the protein is, the faster it moves towards the anode.

-V0
+V0

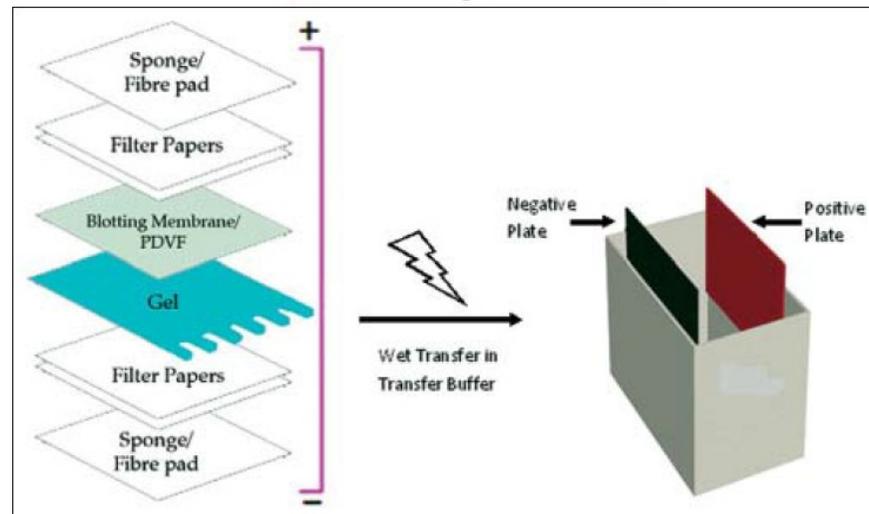


Gel percentage depends on size of protein

4-40 kDa	20%
12-45 kDa	15%
10-70 kDa	12%
15-100 kDa	10%
25-200 kDa	8%

Transfer proteins from the gel to the membrane

Prepare transfer buffer; assemble transfer stack



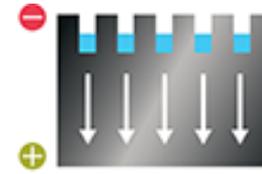
Dia 2

Western blot das fracções nuclear e citosólica

Bloqueio, e incubação com Ac primário



Step 1
Perform
electrophoresis



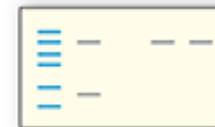
Step 2
Transfer



Step 3
Block with 5%
Milk in PBS-T
1h



Step 4
Incubate with
primary antibodies



Rabbit anti-X
Mouse anti-Y
Streptavidin



Incubação O/N a 4°C

Dia 3

Western blot das fracções nuclear e citosólica

Ac secundário e Autoradiografia



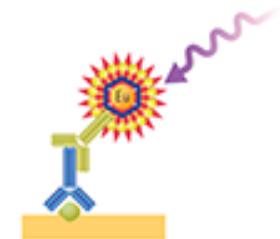
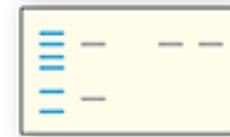
Step 5

Wash with
PBS-T



Step 6

Incubate with
Secondary Ab+HRP
1 h

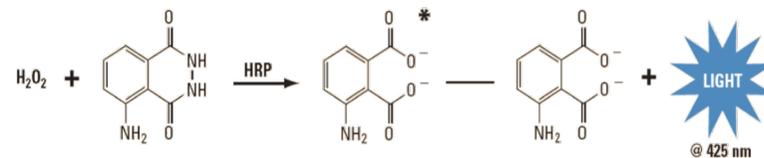


Step 7

Wash with
PBS-T

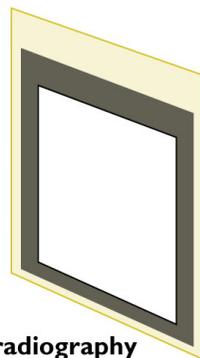


ECL reaction



Antigen bands
visualized

Develop and fix
autoradiograph



Autoradiography

Distribuição tempos experimentais por grupo

2 conjuntos de 9 placas de 35 mm

- **Placas A:** TNF- α
- **Placas B:** TNF- α + MG-132

Tempos	Grupo
15, 45, 75 e 105 min	Grupo 1 (placas A) / Grupo 3 (placas B)
30, 60, 90 e 120 min	Grupo 2 (placas A) / Grupo 4 (placas B)

Distribuição tempos experimentais por grupo

Grupo 1

BSA0	BSA1	BSA2	BSA3	BSA4	BSA5	BSA6		
QAc0	QAc15	QAc30	QAc45	QAc60	QAc75	QAc90	QAc105	QAc120

Grupo 2

BSA0	BSA1	BSA2	BSA3	BSA4	BSA5	BSA6		
QAn0	QAn15	QAn30	QAn45	QAn60	QAn75	QAn90	QAn105	QAn120

Grupo 3

BSA0	BSA1	BSA2	BSA3	BSA4	BSA5	BSA6		
QBc0	QBc15	QBc30	QBc45	QBc60	QBc75	QBc90	QBc105	QBc120

Grupo 4

BSA0	BSA1	BSA2	BSA3	BSA4	BSA5	BSA6		
QBn0	QBn15	QBn30	QBn45	QBn60	QBn75	QBn90	QBn105	QBn120

Avaliação contínua específica

- Mini-teste (30 min)
9h30-10h

1º dia do trabalho prático

14 Março

4 Abril

18 Abril

2 Maio

• **Desempenho laboratorial**

• **Comportamento no laboratório**

Sala 3.2.16