Cross Current International Journal of Medical and Biosciences
Abbreviated Key Title: Cross Current Int J Med Biosci
ISSN: 2663-2446 (Print) & Open Access
DOI: 10.36344/ccijmb.2021.v03i08.002

OPEN ACCESS

Volume-3 | Issue-8 | Nov, 2021 |

Original Research Article

Selection of Detergents Suitable for IBMR3 (Mab) using Balb/c Mouse Muscle

Qutaiba K. J. Alrawi[1*], Nada, S. Alzubydy[2]
[1]Laboratory Department, Surman Medical Technology College, Sabratha University, QFXR+PHC, Sabratah, Libya
[2]Laboratory Department, Faculty of Medical Technology for Alzawia University, QP7X+536, Az-Zāwiyah, Libya

***Corresponding author:** Qutaiba K. J. Alrawi
| **Received:** 08.10.2021 | **Accepted:** 19.11.2021 | **Published:** 30.11.2021|

Abstract: Monoclonal antibodies (Mab) and their fragments have been widely used for diagnostic and therapeutic purposes. Monoclonal antibodies IBMR3 hybridoma cells were produced in a previous study. In my study I used four types of detergents to fine the more suitable as the best Lysis buffer for monoclonal anti bodies using Balb/ c mouse tissue muscle. The four detergents includes; NP- 40, Igepal, Chaps and Triton X-100. Detergents were used in the laboratory to solubilize biological macromolecules such as proteins. These are none denaturing solvents; they also increase emulsification and solubilization, act as solubilize membrane proteins in their native state. The mouse samples were lysed in different lysis buffer detergents, the extracting protein where subjected on the SDS-PAGE electrophoresis, the separated protein bands were transferred to PVDF/ Polyvinylidene difluoride membrane for Immunoblotting technique. The immunblot were subsequently subjected to densitometry analysis to get the value of molecular weight, peak height and raw volume of the protein band. The results of muscle protein concentration of Blab/c mouse after using standard methods were shown (NP-40, 3.214 µg / µl), (Igepal, 3.647 µg / µl), (CHAPS, 3.925 µg / µl and Triton X-100, 4.214 µg / µl). The highest concentration of the muscle protein was obtained from using Triton X-100, followed by CHAPS, then by Igepal and in NP- 40.

Keywords: Detergents of NP- 40, Igepal, Chaps and Triton X-100, IBMR3 Mono Clonal anti bodies, SDS-PAGE, PVDF membrane.

INTRODUCTION

Monoclonal antibody

Monoclonal antibody (Mab) is a single type of antibody. Cell line fusion between stimulated B-cell with myeloma cell which produce hybridoma cell. The (Mab) then will produce by cloning of a single hybridoma or single parent cell line; sometimes naturally, myeloma cells produce single Mab (Hawkins *et al*., 1992).

The uses of monoclonal antibodies (Mabs) have been accepted for diagnosis and therapeutic medical indications, especially in oncology (Van Dongen *et al*., 2007: Abouzied *et al*., 1993; Cheung *et al*., 2002; Emanuel *et al*., 2000). Monoclonal antibodies can be used for the diagnosis of a specific antigen protein in a cancer cell line, normal cell line, normal or cancerous organs, bacteria, virus, parasite, food and blood (Mat,2004).

SDS- PAGE has common steps for any kind of starting sample that is protein is to be extracted followed by electrophoresis, transfer to PVDF, immunoblotting and using labeled IBMR3 Mabs.

CHAPS is the abbreviation for the chemical formula 3-[(3-Cholamidopropyl) Dimethyl ammonia] -1-propanesulfonate (CAS No. 75621-03-3).

NP-40 is Tergitol type NP- 40, which is (nonyl phenoxyl poly ethoxyl ethanol) recently replaced Nonidet P-40 with Igepal CA-630, which is described as a "nonionic, non-denaturing detergent and Triton-X (C14H22O) (C2H4O).

In addition to quantification of protein MW, peak height, raw volume we also calculated the protein concentrations. These applications have supported the current study by the molecular weight as the degree of expression of molecular weight may help in diagnosis

Quick Response Code

Journal homepage:
https://crosscurrentpublisher.com

Citation: Qutaiba K. J. Alrawi & Nada, S. Alzubydy (2021). Selection of Detergents Suitable for IBMR3 (Mab) using Balb/c Mouse Muscle. *Cross Current Int J Med Biosci*, *3*(8), 75-82.

of pathogenic antigens in any pathogenic microorganisms.

Objective:

The aim of this test was to find the best Lysis buffer. The more bands or more height peak with more protein density in raw volume meant the best Lysis buffer.

METHODOLOGY

Material and Methods

Four types of detergents (NP- 40, Igepal, Chaps, and Triton X-100) were used to lyses muscles of Balb/c mouse then separated on Sodium dude sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) in Figure 1; then transfer to PVDF membrane as in figure 2, then Immunoblotting was analyzed in densitometry.

Muscle samples were taken from Balb/c mouse powdered under liquid nitrogen for the preparation of four samples.

Muscle tissues samples were all ground under liquid nitrogen using a pestle and mortar. The samples were either stored in liquid nitrogen or prepared for SDS-PAGE after treatment in Lysis buffer (Shapiro, *et al*., (1967).

Detergents were used in the laboratory to solubilize biological macromolecules such as proteins. These are none denaturing solvents; they also increase the solubilization and emulsification.

Preparation of 1 ml Lysis buffer (RIPA) Buffer:

5X Buffer (Tris- EDTA)	200 µl
5X NaCl	200 µl
5 X SDS ((Luryl sulffate)	200 µl
5X DOC (deoxycholic acid)	200 µl
5X Igepal CA 630	200 µl
Protease inhibitor cocktail	10 µl
Final volume	**1 ml**

https://www.bethyl.com/content/RIPA-Lysis-protocol

1ml of RIPA Lysis buffer enough to extraction (5-20) mg of grinding tissue sample or enough for (10^6 -10^7) cells, the Lysis buffer can store at (2-8) Cº.

- CHAPS is the abbreviation for the chemical formula 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CAS No. 75621-03-3).
- NP-40 is Tergitol-type NP- 40, which is (nonyl phenoxyl poly ethoxyl ethanol) recently replaced Nonidet P-40 with Igepal CA-630, which is described as a "nonionic, non-denaturing detergent and Triton-X (C14H22O (C2H4O).

The first sample of 40 mg was subjected to lysis in 500 µl of lysis buffer using sigma kit (MCL -1 Lot 085 k 4002) which contained 5% Igepal detergent. The other lysis buffers were omitted. The Igepal were replaced with other surfactant of the same percent like Nanodate P– 40 (Tergitol), CHAPS & TritonX-100 as shown in Table 1.

The extracts of protein samples were then separated on SDS- PAGE gel electrophoresis 12 %, resolving gel, the gel images were consequently transfer to PVDF (Towbin, *et al*., 1979), Immunoblotting membrane using labeled IBMR3 Mabs, After that the membrane were subjected to densitometry analysis using bioimaging machine which will facilitate to measure the molecular weights, peak height and raw volume of the protein band for the muscle of the mouse (Burnette, 1981). The concentration of each sample was quantified using 2D Quant Kit /Lot 0207-04/Amersham biosciences using 15µl from protein muscle sample and the absorbance read a spectrophotometer.

Table 1: Explains the four different detergents using in Mouse Muscle Lysis buffer

		Components' of Different Lysis buffer					
No	**Detergent**	**Tris EDTA**	**NaCl**	**SDS-PAGE**	**Deoxycholic acid**	**Protease inhibitor cocktail**	**Detergents %**
1	NP- 40	100µl	100µl	100µl	100µl	5 µl	Nanodate - 40S Tergitol 5 %
2	Igepal	100µl	100µl	100µl	100µl	5 µl	Igepal 5 %
3	Chaps	100µl	100µl	100µl	100µl	5 µl	Chaps 5%
4	Tritonx-100	100µl	100µl	100µl	100µl	5 µl	Triton-X 100 5%

5% = 100µl to prepare 500 ml of Lysis buffer

RESULTS

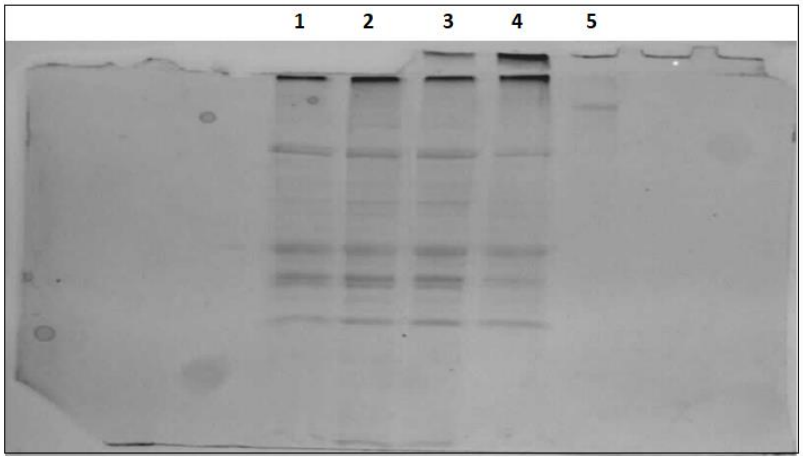

Figure 1: Gel electrophoresis (using Balb/c mouse muscle protein samples) 1- NP-40 2- Igepal 3- CHAPS 4- Triton X-100 5- protein marker

Densitometry results

Muscle molecular weights

PVDF membrane were subjected in densitometry machine as in Figure 2 showed the bands of four Balb/c mouse muscle samples lysed in different lysis buffer **(**NP- 40**,** Igepal**,** CHAPS**,** and Triton X-100**)**.

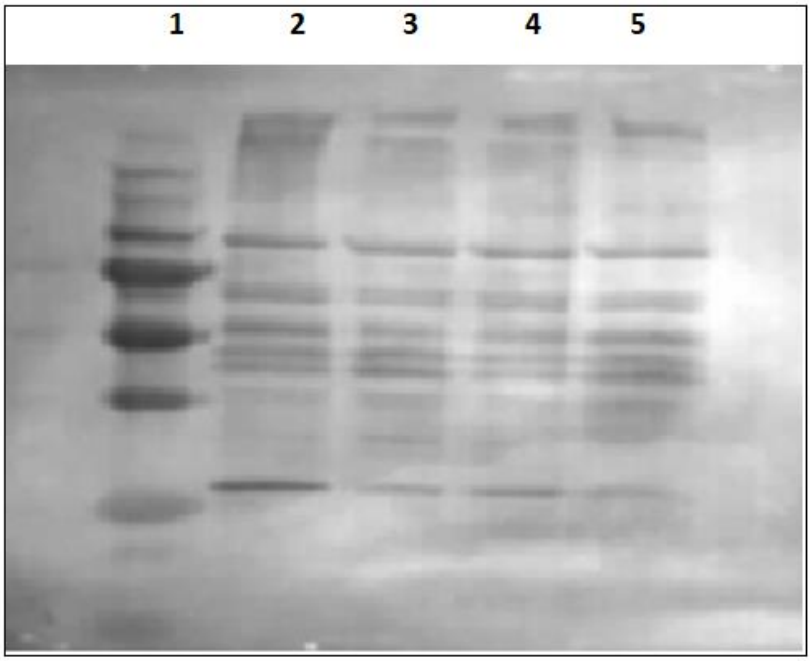

Figure 2: PVDF membrane 1- protein marker 2- TritonX-100 3- CHAPS 4- Igepal 5 – NP-40

After equal volume and concentrations of Balb/c mouse muscle protein samples were lysed with different lysis buffer and sample proteins were separated by SDS-PAGE, transferred on to PVDF membrane, and probed with mab IBMR3, than read the results (Sheen, and Ali-Khan, 2005).

1- Concentration results

The results of muscle protein concentration of Blab/c mouse after using standard methods were shown in Table 2 (NP-40, 3.214 µg / µl), (Igepal, 3.647 µg / µl),(CHAPS, 3.925 µg / µl and Triton X-100, 4.214 µg / µl). The highest concentration of the muscle protein was obtained from using Triton X-100, followed by CHAPS, then by Igepal and in NP- 40**.**

Table 2: The concentration and OD reading for each Balb/c mouse muscle sample in different Lysis buffer

No	Lysis buffer	OD.reading	Conc. µg /15µl	Conc. µg / 1 µl	Con. sample use in SDS-PAGE
1	NP- 40	0.415	48.216	3.214	2µg / µl
2	Igepal	0.376	54.716	3.647	2µg / µl
3	Chaps	0.351	58.883	3.925	2µg / µl
4	Triton X-100	0.323	63.216	4.214	2µg / µl

Protein quantification of Balb/c mouse muscle

Table 3: O.D reading for BSA samples

Number Of sample	BSA volume/ µl	Concentration µg	O.D. Spectrophotometer reading
1	0	0	0.71
2	5	10	0.644
3	10	20	0.577
4	15	30	0.523
5	20	40	0.471
6	25	50	0.408

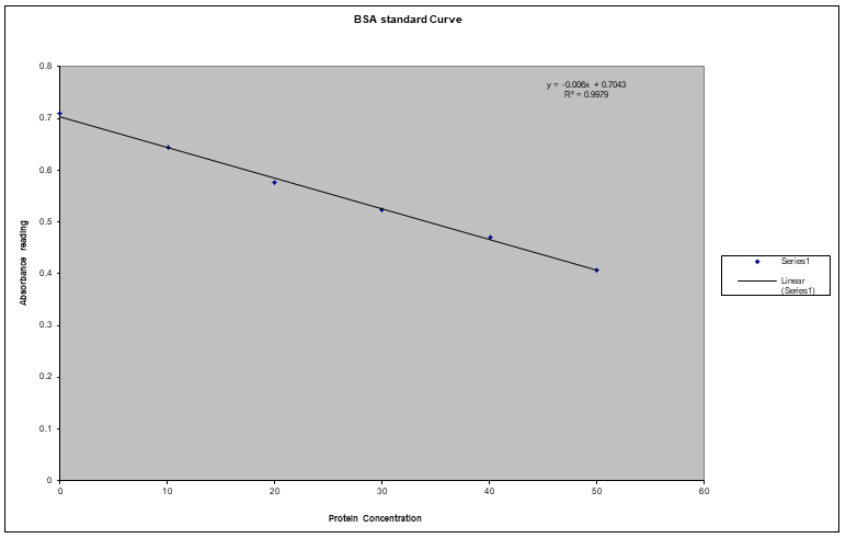

Figure 3: BSA standards curve liner
O.D. = Optical density **BSA =** Bovine serum albumin

Densitometry Results

Muscle molecular weights

PVDF membrane in Figure 2 showed the bands of four Balb/c mouse muscle samples lysed in different Lysis buffer (NP- 40, Igepal, CHAPS, and Triton X-100).

The bands were analysed by densitometry as shown in Tables 4, 5, 6, and 7. The first reading of molecular weight in Table 4 for the lane 2 (TritonX-100) of the seven bands revealed different significant bands and the readings of MW were (96.24, 56.87, 52.92, 46.51, 37.90, 32.91, 27.36) kDa respectively for the IBMR3 Ag.

While the second readings in table 5 of lane 3 for the muscle with (CHAPS buffer) were MW(89.99, 77.19, 64.29, 51.65, 45.68, 42.24, 36.83, 32.03, 26.87, and 22.07) kDa for the IBMR3 Ag respectively with ten bands were shown in Table 5.

The third reading of lane 4 for the muscle using (Igepal buffer) were (87.44, 74.39, 4962.75, 50.41, 44.86, 36.50, 32.61, 26.63, 22.96) kDa of IBMR3 Ag respectively for nine significant bands as shown in Table 6.

The fourth reading of lane 5 for the muscle using (NP-40 buffer) were (88.28, 62.24, 49.70, 41.74, 33.21, 26.39, 23.41) kDa of IBMR3 Ag respectively for seven different significant bands as shown in Table 7. All reading were taken by bioImaging machine of molecular weight.

Figures 2 represent four graphs of muscle Balb/c mouse lysed in different lysis buffer Triton – X densitometry, CHAPS densitometry, Igepal densitometry and NP- 40 densitometry.

The graph a from figure 2 (Triton – X) recorded in table 4 , showed seven peak heights, the highest one was (1082. 60) in 7th height, followed by

the second peak height was (991.52) in 3th height, the third reading was (925.41) in 4th height, the fourth peak heights was (859.88) in 1st height, the fifth was (850.238) in 2nd height, the sixth was (542.69) in 5th height and the seventh was the lowest peak height (426.79) in 6th height.

The graph b/ CHAPS from figure 2 recorded ten peak heights in table 5, the highest peak height was (789.58) in 6th height, the second reading was (750.948) in 5th height, the third reading was (644.116) ist height, the fourth reading was (561.218) in 4th height, the fifth reading was (499.741) in 3th height , the sixth reading was (472.612) in 7th height , the seventh reading was (414.028) in 8th height, the eighth reading was (412.532) in 9th height, the ninth reading was (236.900) in 2nd height while the lowest reading was (119.857) in 10th height.

The graph c / Igepal from figure 2 recorded nine peak heights in Table 6 shows the 50 highest reading of (700.11) in first height, the second reading was (677.36) in 4th height, the third reading was (635.84) in 3th height , the fourth reading was (587.34) in 5th height, the sixth reading was (416.21) in 7th height , the seventh reading was (405.58) in 6th height , the eighth reading was (347.87) in 2th height while the lowest reading was (321.52) in 9th height.

The graph d / NP- 40 shows, from figure 2 recorded seven peak heights in The table 7, shows the first highest reading was (876.77) in 4th height, the second reading was (854.16) in 3th height, the third reading was (678.13) 1st height, the forth reading was (610.92) in 2nd height, the fifth reading was (562.79) in 5th height, the sixth reading was (406.47) in 6th height and the seventh reading was (246.20) in 7th height was the lowest one.

Muscle raw volume (protein band concentration)

The results of four muscle samples with different Lysis buffer are shown in Tables 4, 5, 6, and 7.

In Table 4 for Triton- X100 have seven raw volumes, the more raw volume was (1274723.50) in band four while the lowest one was (196259.31).

The second raw volume for Chaps in Table 5 has ten raw volume, the more raw volume in band sex (676253.50) while the lowest raw volume was found to be (69083.33) in band number ten.

Moreover third raw volume for the Igepal in Table 6 has nine raw volumes, the more raw volume was (669152.81) in band number one and the lowest was (185909.67) in band number nine.

The fourth raw volume for NP-40 in Table 7, the more raw volume (1292163.00) was in band number four and the lowest raw volume (136168.94) was in band number seven.

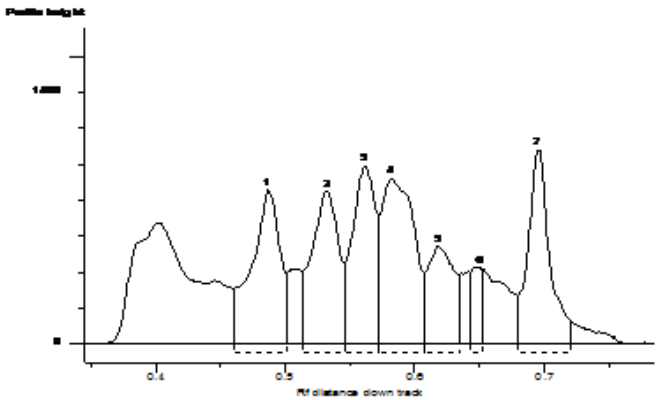

Figure 2a: Densitometry graph of muscle Balb/c mouse lyses in (triton- X Lysis buffer

Track 2 – Triton – X densitometry**)**

Table 4: Track 2 Triton –X 100 protein bands analysis

Lane 2			
Number	**Molecular weight**	**Peak height**	**Raw volume**
1	96.24	859.882	1056838.75
2	65.87	850.238	977030.88
3	52.92	991.520	999085.63
4	46.51	925.418	1274723.50
5	37.90	542.695	627764.00
6	32.91	426.794	196259.31
7	27.36	1082.608	995747.63

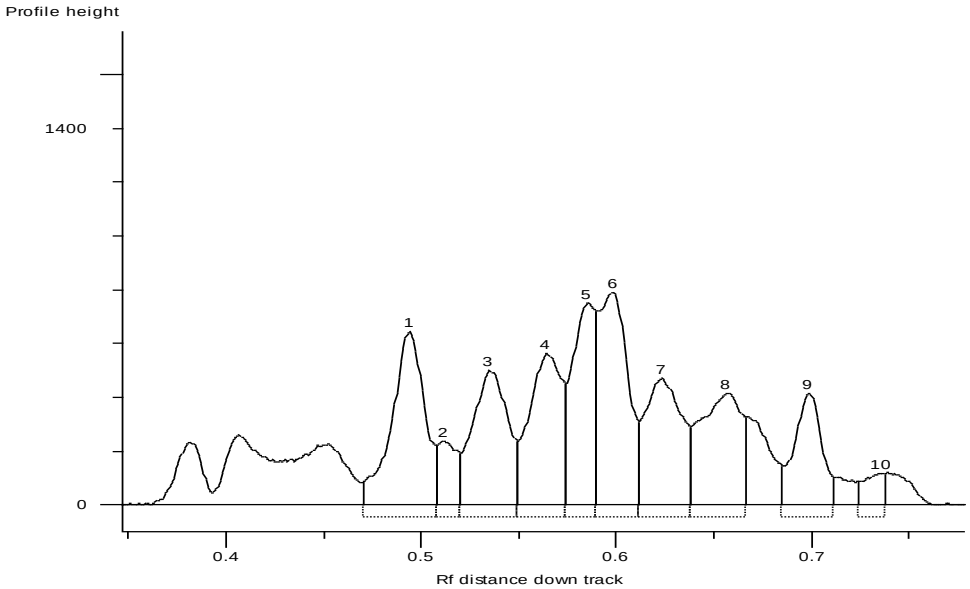

Figure 2b: Densitometry graphs of muscle Balb/c mouse lyses in Chaps Lysis buffer

Track 3 chaps densitometry

Table 5: Track 3 CHAPS protein bands analysis

Track 3			
Number	**Molecular weight**	**Peak height**	**Raw volume**
1	89.99	644.116	631713.38
2	77.19	236.900	130414.77
3	64.29	499.741	522357.94
4	51.65	561.218	541721.50
5	45.68	750.948	519495.34
6	42.24	789.581	676253.50
7	36.83	472.612	517589.78
8	32.03	414.028	501472.72
9	26.87	412.532	341354.16
10	22.07	119.857	69083.33

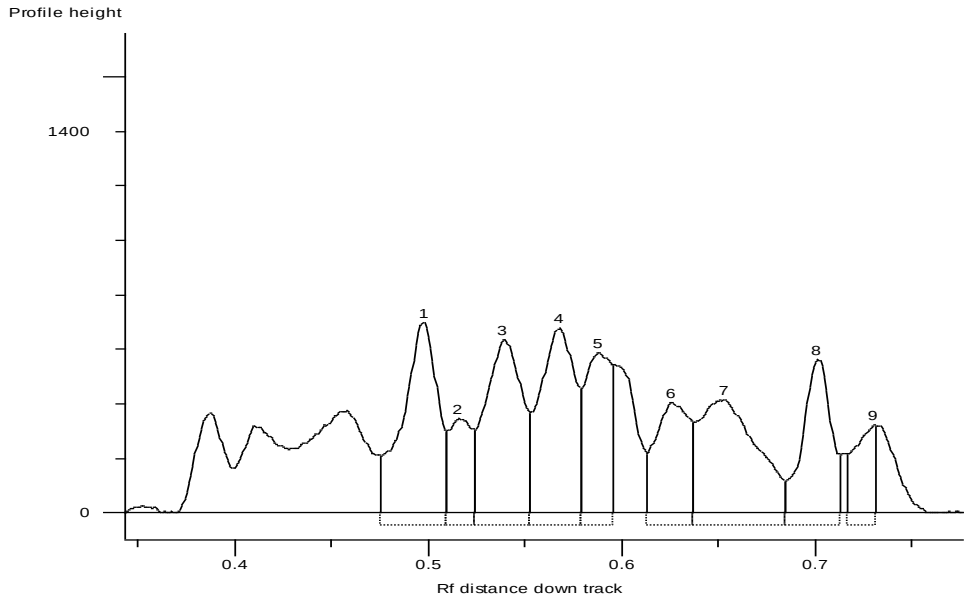

Figure 2.C: Densitometry graphs of muscle Balb/c mouse lyses in Igepal Lysis buffer

Track 4 Igepal densitometry

Table 6: Track 4 Igepal protein bands analysis

Lane 4			
Number	**Molecular weight**	**Peak height**	**Raw volume**
1	87.44	700.115	669152.81
2	74.39	347.875	207969.14
3	62.75	635.843	640212.81
4	50.41	677.362	636835.38
5	44.86	587.349	427780.78
6	36.50	405.582	368466.38
7	32.61	416.212	652074.31
8	26.63	564.527	434222.09
9	22.96	321.520	185909.67

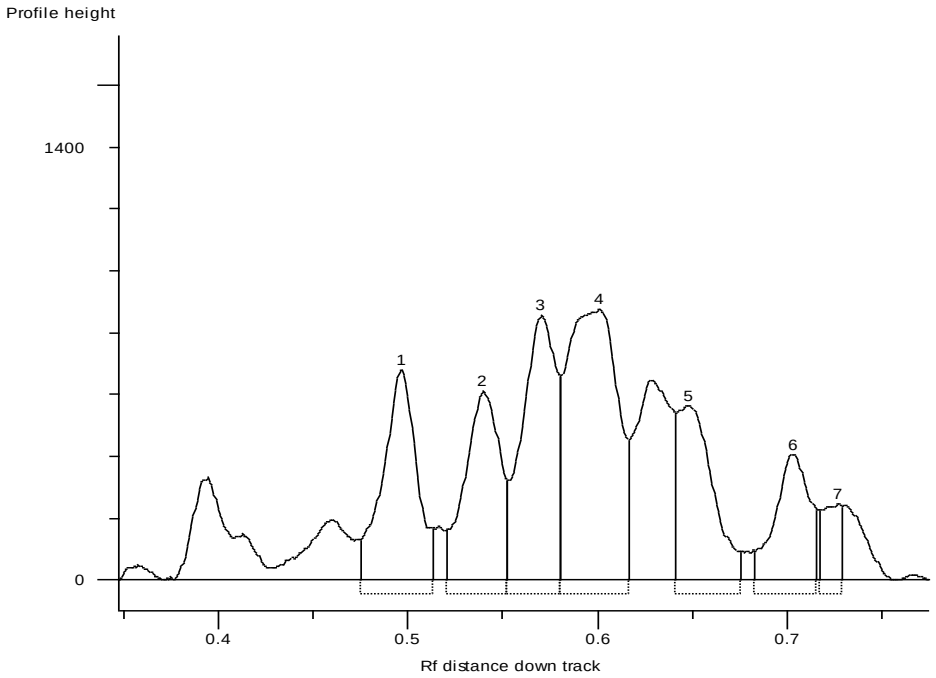

Figure 2d: Densitometry graphs of muscle Balb/c mouse lyses in NP-40 lysis buffer

Track 5 NP- 40 densitometry

Table 7: Track 5 NP- 40-protein bands analysis

lane 5			
Number	**Molecular weight**	**Peak height**	**Raw volume**
1	88.28	678.133	671262.81
2	62.24	610.920	631665.63
3	49.70	854.164	863570.44
4	41.74	876.777	1292163.00
5	33.21	562.794	585592.06
6	26.39	406.474	402668.94
7	23.41	246.209	136168.94

Table 8: Comparison for the highest results of muscle Balb/c mouse protein samples using four different Lysis buffers

No	Sample	Molecular weight /kDa	Peak height	Raw volume	No. of bands
1	**Chaps**	89.99	789.581	676253.50	10
2	**Igepal**	87.44	700.115	669152.81	9
3	**Triton- X**	27.36	1082.608	995747.63	7
4	**NP- 40**	88.28	876.777	1292163.00	7

DISCUSSION

Through Table No. 8, showing the results of densitometry analysis for the three types of mouse muscle samples, we notice that there is a closeness in the molecular weight of the muscle models, except for triton-x, which is considered to be of low molecular weight compared to the rest.

While the peak height is the highest in the Triton-X Lysis buffer model with 1082.608, while the raw volume number 1292163.00 is the highest in the NP-40 model.

Whereas the Chaps sample has 10 bands. 10 bands mean, that Chaps has more ability to dissolve the cell wall and release more antigenic proteins, which were associated with IBMR3 Mab, which appeared in the Chaps band more than helps in diagnostics.

REFERENCES

- Hawkins, R. E., Llewelyn, M. B., & Russell, S. J. (1992). Adapting antibodies clinical use. *BMJ*, 305, 1348-1352.
- Van Dongen, G. A., Visser, G. W., Lub-de Hooge, M. N., De Vries, E. G., & Perk, L. R. (2007). Immuno-PET: a navigator in monoclonal antibody development and applications. *The oncologist*, *12*(12), 1379-1389.
- Abouzied, M. M., Azcona-Olivera, J. I., Yoshizawa, T., & Pestka, J. J. (1993). Production of polyclonal antibodies to the trichothecene mycotoxin 4, 15-diacetylnivalenol with the carrier-adjuvant cholera toxin. *Applied and environmental microbiology*, *59*(5), 1264-1268.
- Cheung, H. Y., Chan, K. M., Ng, T. B., & Cheng, C. H. (2002). Production of a polyclonal antibody against recombinant goldfish prolactin and demonstration of its usefulness in a non-competitive antigen-capture ELISA. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *131*(1), 37-46.
- Emanuel, P. A., Dang, J., Gebhardt, J. S., Aldrich, J., Garber, E. A., Kulaga, H., ... & Dion-Schultz, A. (2000). Recombinant antibodies: a new reagent for biological agent detection. *Biosensors and Bioelectronics*, *14*(10-11), 751-759.
- Burnette, W. N. (1981). Western blotting: electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radio iodinated protein A, *Analytical Biochemistry*, 112(2), 195-203.
- Towbin, H., Staehekin, T., & Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings National Academy of Sciences of the United States of America*, 76(9), 4350-4354.
- Hara, Y., & Mat, I. B. (2004). Differential expression of IBMR3 antigens in normal and transformed cells. *Medimond International Proceedings: Immunology*, E7 18C4844, 229- 233.
- Shapiro, A. L., Vinuela, E.and Maizel, J. V. Jr. (1967). Molecular Weight Estimation of Polypeptide Chains by Electrophoresis in SDS Polyacrylamide Gels. *Biochemical and Biophysical Research Communications*, 28(5), 815-820.
- Sheen, H., & Ali-Khan, Z. (2005) Protein sample concentration by repeated loading onto SDS–PAGE. *Analytical Biochemistry*, (343), 338–340.
- https://www.bethyl.com/content/RIPA-Lysis-protocol.